DESIGN AND OPTIMIZATION OF ZIDOVUDINE NIOSOMES

Dissertation

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CERTIFICATE

This is to certify that the dissertation entitled **"DESIGN AND OPTIMIZATION OF ZIDOVUDINE NIOSOMES**" submitted by **Mr.A.KARTHIKEYAN** to The Tamilnadu Dr.M.G.R. Medical University, Chennai, in partial fulfillment for the award of Master of Pharmacy in Pharmaceutics at **K.M. COLLEGE OF PHARMACY, MADURA**I, is a bonafide work carried out by him under my guidance and supervision during the academic year **2011-2012.**

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"Only that science is a great and the best of all sciences;
The study of which frees man from all kind or miseries."

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It is my duty to express my thanks to god in every moment of my life including this time of project work.

A.KARTHIKEYAN

DEDICATED

TO

MY BELOVED

PARENTS

&

SISTERS

1. INTRODUCTION

1.1. Novel drug delivery system [1]

In the past few decades, considerable attention has been focused on the development of novel drug delivery system (NDDS). The NDDS should ideally fulfil two prerequisites. Firstly, it should deliver the drug at a rate directed by the needs of the body, over the period of treatment. Secondly, it should channel the active entity to the site of action. Conventional dosage forms including prolonged release dosage forms are unable to meet none of these. At present, no available drug delivery system behaves ideally, but sincere attempts have been made to achieve them through various novel approaches in drug delivery.

Merits of novel drug delivery system

- ✓ Reduces the total amount of drug administered over the period of drug treatment, so it reduces the systemic and local side effects.
- ✓ Targeting of the drug molecule towards the tissue (or) organ reduces the toxicity to the normal tissues.
- ✓ Pulsatile and pH dependent systems release the drug whenever the body demands.
- ✓ Improved patient compliance resulting from the reduction in the frequency of doses required to maintain the desired therapeutic response.
- ✓ Devoid of gastrointestinal tract degradation and first pass metabolism.

Limitations of novel drug delivery system

- ✓ To reach previously inaccessible domains e.g. intracellular site, bacteria, viruses parasites etc.
- ✓ To protect the drug and the body from one another until it reaches at the desired site of action.
- ✓ To control the frequency and rate of drug delivery at the pharmacological receptor.
- ✓ Reduction in the drug dose and side effects.
- ✓ Exclusive drug delivery to the specific cells or diseased site in the body.

1.1.1. Targeted drug delivery system [3,4]

Drug targeting can be defined as the ability to direct a therapeutic agent specifically to desired site of action with little or no interaction with nontarget tissue. Targeted drug delivery system means accumulation of pharmacologically active moiety at desired target site in therapeutic concentration at the same restricting its access to normal cellular lining, thus minimizing therapeutic index. In site specific targeted drug delivery, active drug is delivered to preselected and very specific compartments with maximum activity while reducing the concentration of drug to normal cells. The drug can be targeted to intracellular sites, virus cells, bacterial cells and parasites using different scientific strategies have proven very effective. Minimum distribution of the parent drug to the non target sites with higher and effective concentration at the targeted site certainly maximize the benefits of targeted drug delivery.

Properties of ideal Targeted drug delivery

- It should possess controllable and predictable rate of drug release.
- Carriers used should be inert or should have zero therapeutic value.
- Carriers should be biodegradable and easily eliminated from the body.
- The preparation of drug delivery system should be easy or reasonably simple, reproductive and cost effective.
- Should be nontoxic and physicochemical stable *in vivo* and *in vitro*.
- Drug release should not affect the drug distribution

Targeted drug delivery systems are preferred under following situations,

- Stability of the drug is poor.
- Insoluble drugs.
- Drugs which are having poor absorption,
- Drugs with low specificity and low therapeutic index.
- Drugs with short biological half life.
- Drugs with large volume of distribution.

1.1.2. Types of drug targeting [4]

Passive Targeting

In this type of targeting the particle system is captured by physiological mechanism such as filtration or macrophage (Reticulo endothelial system) sequestration. (i.e) drug targeting occurs because of the body's natural response to physicochemical characteristics of the drug or drug carrier system. It is concentration dependent, so external energy is not necessary.

Inverse Targeting:

To achieve inverse targeting, RES normal function is suppressed by preinjecting large amount of blank colloidal carriers or macromolecules like dextran sulphate. This approach leads to saturation of RES and suppression of defense mechanism. This type of targeting is a effective approach to target drug(s) to non-RES organs.

Active targeting:

Surface modification technique is used to achieve active targeting. In this approach active ingredient is attached with the surface of carrier sytem such as monoclonal antibodies or carbohydrates like glucose and galactose. The drug reaches to specific site on the basis of modification made on its surface rather than natural uptake by RES. Active targeting is classified into three,

i) First order targeting

It involves distribution of drug carrier system to capillary bed of target site or organ. For example lymphatic's, peritoneal cavity, plural cavity, cerebral ventricles, etc.

ii) Second order targeting

It involves delivery of drug to special cells such as tumor cells or kupffer cells in lives.

iii) Third order targeting

Third order targeting is essential for gene delivery and exogenous DNA to the nucleus. Targeting is based on the structure with in a cell. The active targeting more specific for kupffer cells of the liver and parenchymal cells like hepatocytes.

Dual Targeting

In this targeting approach carrier molecule itself have their own therapeutic activity and thus increase the therapeutic effect of drug. For example, a carrier molecule having its own antiviral activity can be loaded with antiviral drug and the net synergistic effect of drug conjugate was observed.

Double Targeting

When temporal and spatial methodologies are combined to target a carrier system, then targeting may be called double targeting. Spatial placement relates to targeting drugs to specific organs.

Combination targeting

Combination targeting is for site specific delivery of proteins and peptides. The targeting systems are equipped with carrier and polymer. This method is more specific for gene therapy.

1.1.3. Drug delivery carriers [4,9]

Carriers are used to achieve targeted drug delivery. Carrier is one of the special molecule or system essentially required for effective transportation of loaded drug up to the preselected sites. They are engineered vectors, which retain drug inside or onto them either via encapsulation and/ or via spacer moiety and transport or deliver it into vicinity of target cell.

Some carrier based drug delivery systems are,

- Microspheres and micro capsules
- Nanoparticles
- Monoclonal antibodies
- Prodrugs
- Resealed erythrocytes
- Artificial cells
- Neutrophils
- Vesicular carriers

1.1.4. Vesicular system – carrier for drug delivery [5]

Vesicles act as the vehicle of choice in drug delivery. Lipid vesicles were found to be of value in immunology, membrane biology, diagnostic techniques, and most recently, genetic engineering. Vesicles can play a major role in modelling biological membranes, and in the transport and targeting of active agents. Vesicular drug delivery system has some of the advantages,

- Like other targeted drug delivery systems, it prolongs the existence of the drug in systemic circulation, and perhaps, reduces the toxicity due to the delivery of drug directly to the site of infection.
- Both hydrophilic and lipophilic drugs can be incorporated.
- Delays elimination of rapidly metabolizable drugs and thus function as sustained release systems.

Some important vesicular drug delivery systems are,

- Liposomes
- Sphinosomes
- Transferosomes
- Pharmacosomes
- Niosomes

i) Liposomes

Liposomes are simple microscopic vesicles in which lipid bilayer structures are present with an aqueous volume entirely enclosed by a membrane, composed of lipid molecule. There are a number of components present in liposomes, with phospholipid and cholesterol being the main ingredients. The type of phospholipids includes phosphoglycerides and sphingolipids, and together with their hydrolysis products.

ii) Sphinosomes

Liposome has some stability problems. Liposomal phospholipid can undergo chemical degradation such as oxidation and hydrolysis. The hydrolysis may be avoided altogether by use of lipid which contains ether or amide linkage instead of ester linkage (such are found in sphingolipid) or phospholipid derivatives with the 2-ester linkage replaced by carbomoyloxy function**.** Thus sphingolipid are been

nowadays used for the preparation of stable liposomes known as sphingosomes. Sphingosome may be defined as "concentric, bilayered vesicle in which an aqueous volume is entirely enclosed by a membranous lipid bilayer mainly composed of natural or synthetic sphingolipid.

iii) Transferosomes

Transferosomes was introduced for the effective transdermal delivery of number of low and high molecular weight drugs.It consist of both hydrophilic and hydrophobic properties,high deformablity gives better penetration of intact vescicles. A transferosomes, in functional terms, may be described as lipid droplets of such deformability that permits its easy penetration through the pores much smaller than the droplets size. They protect the encapsulated drug from metabolic degradation. In thermodynamics terms this typically corresponds to an aggregate in the quasi-metastable state, which facilitates the formation of highly curved bilayers.

iv) Pharmacosomes

The term pharmacosome comprises of two main parts - Pharmacon (active principle) and some carriers (Goymann and Hamann, 1991) Vaizogle and Speiser (1986) postulated that amphipathic drug can self assemble to form vesicle and these vesicles are termed as pharmacosomes. Drug covalently bound to lipid may exist in a colloidal dispersion as ultrafine, micelles or hexagonal aggregates which are known as pharmacosomes.

v) Niosomes

Niosomes or non-ionic surfactant vesicles are microscopic lamellar structures formed on admixture of non-ionic surfactant of the alkyl or dialkyl polyglycerol ether class and cholesterol with subsequent hydration in aqueous media. Niosomes are promising vehicle for drug delivery and being non-ionic, Niosomes are unilamellar or multilamellar vesicles formed from synthetic non-ionic surfactants. They are very similar to the liposomes. Niosomal drug delivery is potentially applicable to many pharmacological agents for their action against various diseases. Niosomes have shown promise in the release studies and serve as a better option for drug delivery system. The drug is incorporated into niosomes for a better targeting of the drug at appropriate tissue destination.

1.2 Salient features of niosomes [7]

- Niosomes act as alternatives of liposomes. Disadvantages in the liposomes are avoided in this.
- Osmotically active and stable.
- Niosome increases the stability of entrapped drug.
- They can be made to reach the site of action by oral, parenteral as well as topical routes.
- Surfactant used in niosome does not require special conditions.
- Surfactants used in niosomes are biodegrable, biocompatible and non-immunogenic.
- They improve the therapeutic performance of the drug molecules by delayed clearance from the circulation.
- Niosome exhibit flexibility in their structural characteristics (composition, fluidity, size) and can be designed to desired situation.

1.3. Composition of niosomes [10]

Niosomes contain two major component, Cholesterol and Nonionic surfactants. Cholesterol is used to provide rigidity and proper shape to the niosomes. surfactants play a major role in the formation of niosomes. The following non-ionic surfactants are generally used for the preparation of niosomes the spans (span60,40,20,85,80), tweens (tween 20,40,60,80) and brijs (brij 30,35,52,58,72,76). The non ionic surfactants possess a hydrophillic head and a hydrophobic tail.

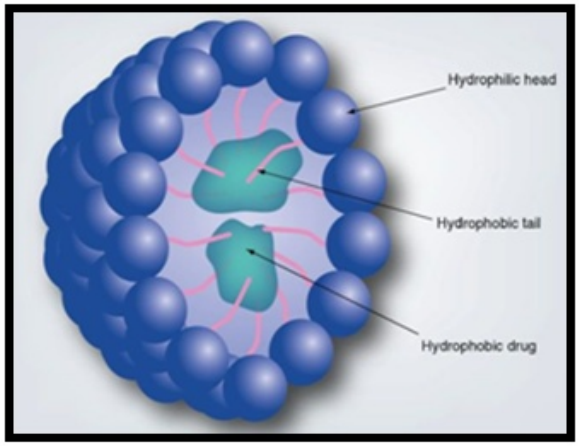


Fig:1 Internal structure of niosome

1.4. Surfactants used in formulation of niosomes [10]

Niosomes are non-ionic surfactant unilamellar or multilamellar vesicles formed from synthetic non-ionic surfactants. The surfactants that are reported to form niosomes are as follows:

1. Ether linked surfactant

These are surfactants in which the hydrophilic hydrophobic moieties are ether linked, polyoxyethylene alkyl ethers with the general formula (CnEOm), where n; i.e. number of carbon atoms varies between 12 and 18 and m; i.e. number of oxyethylene unit varies between 3 and 7.

2. Di-alkyl chain surfactant

Surfactant was used as a principal component of niosomal preparation of stibogluconate and its potential in delivering sodium stibogluconate in experimental marine visceral leishmaniasis has been explored.

$C_{16}H_{33}CH\text{-}O\ [\text{-}CH_2\text{-}CH\text{-}O]_7\text{-}H$
| |
$CH_2\ CHOH$
|
$C_{12}H_{25}\text{-}O$ (mol. Wt. 972)

3. Ester linked

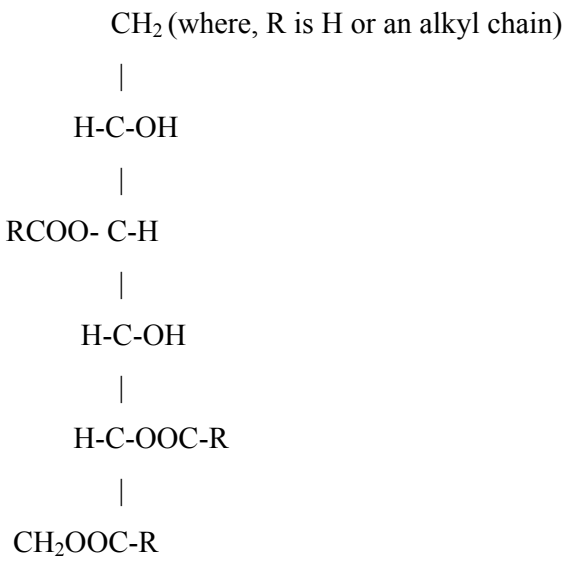
These are the surfactants in which hydrophilic and hydrophobic moieties are ester linked. Ester linked surfactant,

$C_{15}\ H_{31}\ CO\ [O\text{-}CH_2\text{-}CH\text{-}CH]_2\text{-}OH$
|
OH (mol. Wt. 393)

This surfactant was also studied for its use in the preparation of stibogluconate bearing niosomes and in delivery of sodium stibogluconate to the experimental marine visceral leishmaniasis following administration of niosomal system. The commercial sorbitan esters are H-C-OH mixtures of the partial esters of sorbital.

4. Sorbitan Esters:

The typical structural formula of sorbitan ester is,

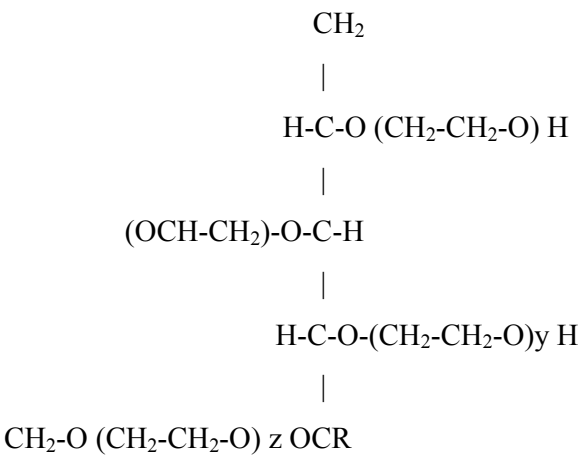


The formula of a representative component is shown above. Sorbitan esters based niosomes bearing methotrexate were prepared and evaluated for pharmacokinetics of the entrapped methotrexate in tumor bearing mice.

5. Poly-sorbates

The typical structural formula of polysorbates is

-



When n = x + y + z + 2 and R is an alkyl chain this series of surfactants has been used to study the pharmacokinetics of niosomal entrapped methotrexate.

1.5. Types of niosomes

1. Small unilamellar vesicles (SUV)

SUV are commonly produced by sonication, and French Press procedures. Ultrasonic electro capillary emulsification or solvent dilution techniques can be used to prepare SUVs. (size -0.025-0.05 μm)

2. Multilamellar vesicles (MUV)

Exhibit increased-trapped volume and equilibrium solute distribution, and require hand-shaking method. They show variations in lipid compositions. (size > 0.05 μm)

3. Large unilamellar vesicles (LUV)

The injections of lipids solubilised in an organic solvent into an aqueous buffer, can result in spontaneous formation of LUV, but the better method of preparation of LUV is Reverse phase evaporation, or by Detergent solubilisation method. (size > 0.10 μm).

1.6. Advantages of niosomes [5,7,8]

- Niosomal vesicle suspension is water–based vehicle. This offers high patient compliance in comparison with oily dosage forms.
- They possess an infrastructure consisting of hydrophilic, amphiphilic and lipophilic moieties together and as a result can accommodate drug molecules with a wide range of solubilities.
- The characteristics of the vesicle formulation are variable and controllable. Altering vesicle composition, size, lamellarity, tapped volume, surface charge and concentration can control the vesicle characteristics.
- The vesicles may act as a depot, releasing the drug in a controlled manner.
- They improve oral bioavailability of poorly absorbed drugs and enhance skin penetration of drugs.
- They can be made to reach the site of action by oral, parenteral as well as topical routes
- They improve the therapeutic performance of the drug molecules by delayed clearance from the circulation, protecting the drug from biological environment and restricting effects to target cells.

- Niosomal dispersion in an aqueous phase can be emulsified in a non-aqueous phase to regulate the delivery rate of drug and administer normal vesicle in external non-aqueos phase.

1.7. Factors affecting formation of niosomes [11,12]

i) Nature of surfactants

Surfactants used for preparation of niosomes must contain a hydrophilic head and hydrophobic tail. The hydrophobic tail may consist of one or two alkyl or perfluoroalkyl groups or in some cases a single steroidal group. The ether type surfactants with single chain alkyl as hydrophobic tail is more toxic than corresponding dialkylether chain. The ester type surfactants are chemically less stable than ether type surfactants and the former is less toxic than the latter due to ester-linked surfactant degraded by esterases to triglycerides and fatty acid *in vivo*. The surfactants with alkyl chain length from C_{12}-C_{18} are suitable for preparation of niosome.

ii) Structure of surfactants

The geometry of vesicle to be formed from surfactants is affected by its structure, which is related to critical packing parameters. On the basis of critical packing parameters of surfactants can predicate geometry of vesicle to be formed. Critical packing parameters can be defined using following equation,

$$\text{CPP (Critical Packing Parameters)} = \frac{v}{lc \times a_0}$$

Where v = hydrophobic group volume,
lc = the critical hydrophobic group length,
a_0 = the area of hydrophilic head group.

From the critical packing parameter value type of miceller structure formed can be ascertained as given below,

If CPP < ½ then formation of spherical micelles,
If ½ < CPP < 1 formation of bilayer micelles,
If CPP > 1 formation of inverted micelles.

iii) Membrane composition

The stable niosomes can be prepared with addition of different additives along with surfactants and drugs. Niosomes formed have a number of morphologies and their permeability and stability properties can be altered by manipulating membrane characteristics by different additives.

iv) Nature of encapsulated drug

The physico-chemical properties of encapsulated drug influence charge and rigidity of the niosome bilayer. The drug interacts with surfactant head groups and develops the charge that creates mutual repulsion between surfactant bilayers and hence increases vesicle size.

v) Temperature of hydration

Hydration temperature influences the shape and size of the niosome. For ideal condition it should be above the gel to liquid phase transition temperature of system. Temperature change of niosomal system affects assembly of surfactants into vesicles and also induces vesicle shape transformation.

1.8. Characterization of niosomes [12,15]

i) Size

Shape of niosome vesicles assumed to be spherical, their mean diameter can be determined by using laser light scattering method. Also diameter can be determined by using electron microscopy, molecular sieve chromatography, ultracentrifugation, photon correlation microscopy and optical microscopy.

ii) Bilayer formation

Assembly of non-ionic surfactants to form bilayer vesicle is characterized by X-cross formation under light polarization microscopy.

iii) Number of lamellae

It can be determined by using NMR spectroscopy, small angle X-ray scattering and electron microscopy.

iv) Membrane rigidity

Membrane rigidity can be measured by means of mobility of fluorescence probe as function of temperature.

v) Entrapment efficiency (EE)

The entrapment efficiency (EE) is expressed as

$$EE = \frac{\text{Amount entrapped}}{\text{Total amount added}} \times 100$$

It is determined after separation of unentrapped drug, on complete vesicle disruption by using about 1ml of 2.5% sodium lauryl sulfate, briefly homogenized and centrifuged and supernatant assayed for drug after suitable dilution. Entrapment efficiency is affected by following factors.

a) Surfactants

The chain length and hydrophilic head of non-ionic surfactants affect entrapment efficiency, such as stearyl chain C_{18} non-ionic surfactant vesicles show higher entrapment efficiency than lauryl chain C_{12} non-ionic surfactant vesicles. The tween series surfactants bearing a long alkyl chain and a large hydrophilic moiety in the combination with cholesterol at 1:1 ratio have highest entrapment efficiency for water soluble drugs. HLB value of surfactants affects entrapment efficiency, such as HLB value of 14 to 17 is not suitable for niosomes, but HLB value of 8.6 has highest entrapment efficiency and entrapment efficiency decreases with decrease in HLB value from 8.6 to 1.7. The entrapment efficiency is affected by phase transition temperature of surfactants, i.e. span 60 exhibits highest entrapment efficiency in series having highest transition temperature (Tc).

b) Cholesterol contents

The incorporation of cholesterol into bilayer composition of niosome induces membrane-stabilizing activity and decreases the leakiness of membrane. Hence, incorporation of cholesterol into bilayer increases entrapment efficiency.

1.9. Comparision of niosome with liposome [6,8]

Niosomes are now widely studied as alternative to liposomes. Liposomes face problems such as, they are expensive, their ingredients like phospholipids are chemically unstable because of their predisposition to oxidative degradation, they require special storage and handling and purity of natural phospholipids is variable. Niosomes do not have any of these problems. Also since niosomes are made of uncharged single-chain surfactant molecules as compared to the liposomes which are made from neutral or charged double chained phospholipids, the structure of niosomes is different from that of liposomes. However Niosomes are similar to liposomes in functionality. Niosomes also increase the bioavailability of the drug and reduce the clearance like liposomes. Niosomes can also be used for targeted drug delivery, similar to liposomes.

Table:2

Carrier System	Size Range	Features	Method of Preparation	Application
Liposomes	25nm-100µm	microscopic vesicles composed of one or more concentric lipid bilayers, separated by water or aqueous buffer compartment	Mechanical Dispersion. solvent dispersion detergent removal etc.	In cancer, malaria, AIDS, lung therapies. As radio diagnostic carrier. As an immunological adjuvant.
Niosomes	10 to 1000 nm	non-ionic surfactant vesicles are bilayered structures	Ether injection, Sonication, REV, microfluidization etc.	Targeting of bioactive agents Delivery of peptide drug In diseases like neoplasia, leishmaniasis

1.10. Methods of preparation of niosomes [8,14,15]

i) Ether injection method

This method provides a means of making niosomes by slowly introducing a solution of surfactant dissolved in diethyl ether (volatile organic solvent) into warm water maintained at 60°C. The surfactant mixture in ether is injected through 14-gauge needle into an aqueous solution of material. Vaporization of ether (volatile organic solvent) leads to formation of single layered vesicles. Depending upon the conditions used the diameter of the vesicle range from 50 to 1000 nm.

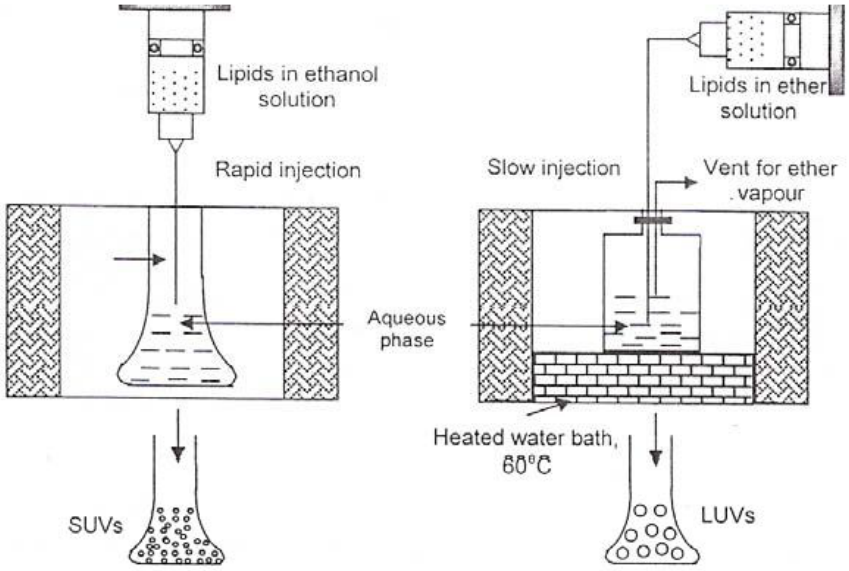


Fig:2 Ether injection method

ii) Thin film hydration technique

The mixture of vesicles forming ingredients like surfactant and cholesterol are dissolved in a volatile organic solvent (diethyl ether, chloroform or methanol) in a round bottom flask. The organic solvent is removed at room temperature (20°C) using rotary evaporator leaving a thin layer of solid mixture deposited on the wall of the flask. The dried surfactant film can be rehydrated with aqueous phase at 0-60°C with gentle agitation. This process forms typical multilamellar niosomes.

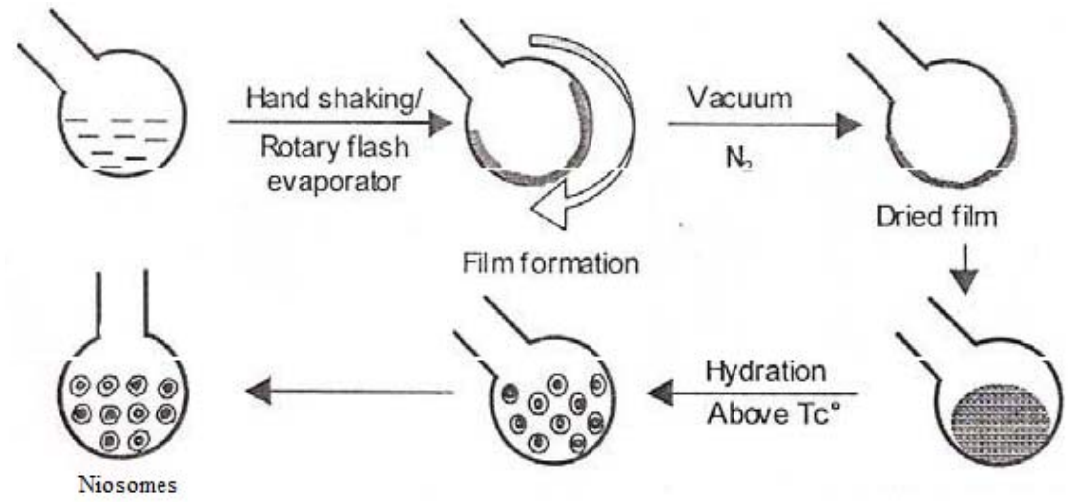


Fig:3 Thin film hydration technique

iii) Sonication method

In this method an aliquot of drug solution in buffer is added to the surfactant/cholesterol mixture in a 10-ml glass vial. The mixture is probe sonicated at 60°C for 3 minutes using a sonicator with a titanium probe to yield niosomes.

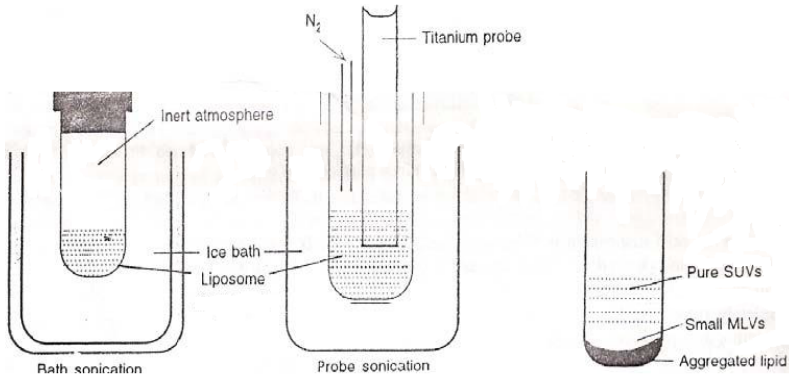


Fig:4 sonication method

iv) Reverse phase evaporation technique (REV)

Cholesterol and surfactant (1:1) are dissolved in a mixture of ether and chloroform. An aqueous phase containing drug is added to this and the resulting two phases are sonicated at 4- 5°C. The clear gel formed is further sonicated after the addition of a small amount of phosphate buffer saline (PBS). The organic phase is

removed at 40°C under low pressure. The resulting viscous niosome suspension is diluted with PBS and heated on a water bath at 60°C for 10 min to yield niosomes.

v) Micro fluidization

It is a recent technique used to prepare unilamellar vesicles of defined size distribution. This method is based on submerged jet principle in which two fluidized streams interact at ultra high velocities, in precisely defined micro channels within the interaction chamber. The impingement of thin liquid sheet along a common front is arranged such that the energy supplied to the system remains within the area of niosomes formation. The result is a smaller size, greater uniformity and better reproducibility of niosomes formed.

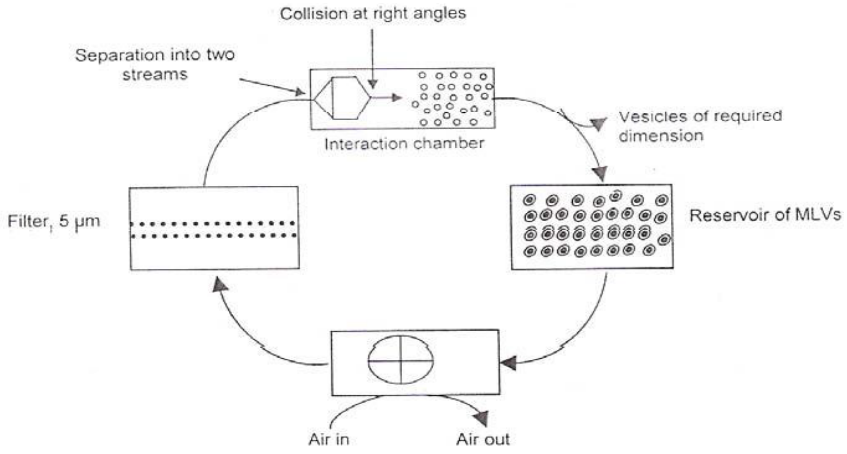


Fig:5 Micro fluidization

vi) Multiple membrane extrusion method

Mixture of surfactant, cholesterol and dicetyl phosphate in chloroform is made into thin film by evaporation. The film is hydrated with aqueous drug polycarbonate membranes, solution and the resultant suspension extruded through which are placed in series for upto 8 passages. It is a good method for controlling niosome size.

vii) Trans membrane pH gradient (inside acidic) Drug Uptake Process (remote Loading)

Surfactant and cholesterol are dissolved in chloroform. The solvent is then evaporated under reduced pressure to get a thin film on the wall of the round bottom flask. The film is hydrated with 300 µM citric acid (pH 4.0) by vortex mixing. The

multilamellar vesicles are frozen and thawed 3 times and later sonicated. To this niosomal suspension, aqueous solution containing 10 mg/ml of drug is added and vortexed. The pH of the sample is then raised to 7.0-7.2 with 1M disodium phosphate. This mixture is later heated at 60°C for 10 minutes to give niosomes.

viii) The "Bubble" Method

It is novel technique for the one step preparation of liposomes and niosomes without the use of organic solvents. The bubbling unit consists of round-bottomed flask with three necks positioned in water bath to control the temperature. Water-cooled reflux and thermometer is positioned in the first and second neck and nitrogen supply through the third neck. Cholesterol and surfactant are dispersed together in this buffer (pH 7.4) at 70°C, the dispersion mixed for 15 seconds with high shear homogenizer and immediately afterwards "bubbled" at 70°C using nitrogen gas.

ix) Formation of niosomes from proniosomes

Another method of producing niosomes is to coat a water-soluble carrier such as sorbitol with surfactant. The result of the coating process is a dry formulation. In which each water-soluble particle is covered with a thin film of dry surfactant. This preparation is termed as Proniosomes. The niosomes are recognized by the addition of aqueous phase at T > Tm and brief agitation. T=Temperature. Tm = mean phase transition temperature.

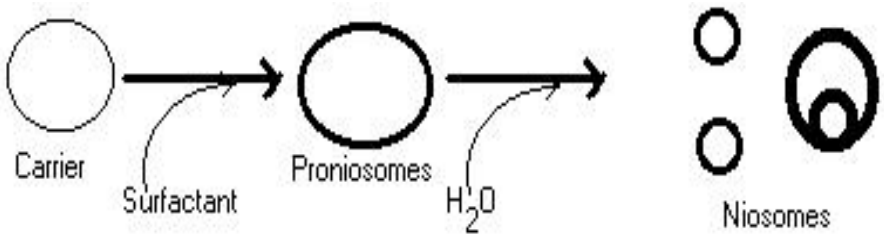


Fig:6 Formation of niosomes from proniosomes

1.11. Methods of separation of unentrapped drug from niosomes [8,11]

The removal of unentrapped solute from the vesicles can be done by various techniques, which include

i) Dialysis

The aqueous niosomal dispersion is dialyzed in dialysis tubing against suitable dissolution medium at room temperature. The samples are withdrawn from the medium at suitable time intervals, centrifuged and analysed for drug content using suitable method (U.V spectroscopy, HPLC etc).

ii) Gel Filtration

The unentrapped drug is removed by gel filtration of niosomal dispersion through a Sephadex- G-50 column and eluted with suitable mobile phase and analyzed with suitable analytical techniques.

iii) Centrifugation

The niosomal suspension is centrifuged and the supernatant is separated. The pellet is washed and then resuspended to obtain a niosomal suspension free from unentrapped drug.

1.12. Stability of niosomes [1]

Physical stability

The niosomes can change their physical characteristics in several ways.

- The particle size may change because of aggregate formation and fusion.
- Occurrence of phase separation of bilayer components, upon storage.
- Leakage of encapsulated material from niosomes.

The changes in particle upon storage of phosphatidyl choline containing niosomes over pharmaceutically relevant time intervals can be minimized by the selection of proper charge inducing agents. Mostly, negatively charged phospholipids (phosphatidyl glycerol) are used to stabilize the niosomes.

The permeability of bilayers is highly dependent on the physico-chemical properties of the bilayer, drug and the temperature. Three categories of drugs can be discerned.

- Highly hydrophilic, non-bilayer interacting drugs.
- Drugs with some lipophilicity.
- Strongly lipophilic drugs.

In category first, the presence of cholesterol in the bilayer of the egg phosphatidyl choline niosomes dramatically reduces the permeability. For gel state bilayers, permeability is low with or without cholesterol. It is clear that if *in vivo* performance allows 'gel state' bilayers to be used, the shelf life of the niosomes in aqueous media with the proper pH might easily meet industrial demands. In the second category, the drug tends to be difficult to keep entrapped over periods of months as long as outside sink conditions prevail. In the third category, strongly lipophilic drugs have high affinity for the bilayer and therefore these drugs stay there over a long period of time, independently of the state of the bilayer.

As the final remark, the presence of hydrolysis or oxidation reduction products can affect bilayer properties. Although, lysophosphatidyl choline is known to be a lipid bilayer solubilizer, the solubilizing effect of lysophosphatidyl choline in degrading niosomes is neutralized by the simultaneous appearance of fatty acids in the bilayer.

The niosomes stored in freeze dried form is preferred for proper *in vivo* performance of niosomes with long term stability. To maintain the particle size distribution after freeze drying-rehydration cycle, a cryoprotectant needs to be added. Usually sugars are used as cryoprotectant.

- The formation of amorphous glass structures during the freeze drying process may avoid mechanical damage inflected by ice crystals. It is recommended to store these cakes below the glass transition temperature.
- The sugars may interact with the polar head groups of the phospholipids and stabilize the membranes when the bilayer stabilizing water is removed by sublimation.

Chemical stability

The stability of niosomes depends on the chemical stability of the lipid components and the bilayer components of niosomes, designed for carrying a drug or phospholipids. Usually, hydrolysis and peroxidation are the two degradation process which occurs with phospholipids. Apart from pH, other experimental conditions like temperature, ionic strength, buffer species, and ultra sonication were reported to influence hydrolysis reactions. Many investigators choose the formation of

lysophosphatidyl choline as a standard measure for the chemical stability to phospholipids. Since, the presence of lysophosphatidyl choline in lipid bilayer greatly enhances the permeability of niosomes, the most important method for minimizing this problem is the proper sourcing of the phospholipid to be used. They should be essentially free from any lyso-phosphatidyl choline to start with and free of any peroxidation of phospholipids produces the formation of cyclic peroxides and hydro peroxides. Peroxidation of the phospholipids may be minimized by a number of ways such as,

- Minimum use of unsaturated phospholipids.
- Use of nitrogen or argon to minimize exposure to oxygen.
- Use of light resistant container.
- Removal of heavy metals (EDTA).

Stability in biological fluids

The inability of niosomes to retain entrapped substances when incubated in blood or plasma has been known for a decade. The instability of niosomes in plasma appears to be the result of transfer of bilayer lipids to albumin and high density lipoproteins. Both lecithin and cholesterol also exchanges with the membrane of red blood corpuscle. Niosomes are most susceptible to high density lipoprotein attack at their gel to liquid crystalline phase transition temperature. The susceptibility of niosomal phospholipids to lipoprotein and phospholipase attack is strongly dependent on niosome size and type. Generally, multilamellar vesicles are most stable whereas small lamellar vesicles are least stable. The bile salts also destabilize the bilayer membrane structure, thereby, leading to release of the entrapped material.

1.13. Application of niosomes [13,15,16]

Drug targeting

One of the most useful aspects of niosomes is their ability to target drugs. Niosomes can be used to target drugs to the reticulo-endothelial system. The reticulo-endothelial system (RES) preferentially takes up niosome vesicles. The uptake of niosomes is controlled by circulating serum factors called opsonins. These opsonins mark the niosome for clearance. Such localization of drugs is utilized to treat tumors in animals known to metastasize to the liver and spleen. This localization of drugs can

also be used for treating parasitic infections of the liver. Niosomes can also be utilized for targeting drugs to organs other than the RES. A carrier system (such as antibodies) can be attached to niosomes (as immunoglobulins bind readily to the lipid surface of the niosome) to target them to specific organs. Many cells also possess the intrinsic ability recognize and bind specific carbohydrate determinants, and this can be exploited by niosomes to direct carrier system to particular cells.

Anti-neoplastic Treatment

Most antineoplastic drugs cause severe side effects. Niosomes can alter the metabolism; prolong circulation and half life of the drug, thus decreasing the side effects of the drugs. Niosomal entrapment of Doxorubicin and Methotrexate (in two separate studies) showed beneficial effects over the unentrapped drugs, such as decreased rate of proliferation of the tumor and higher plasma levels accompanied by slower elimination.

Leishmaniasis

Leishmaniasis is a disease in which a parasite of the genus Leishmania invades the cells of the liver and spleen. Commonly prescribed drugs for the treatment are derivatives of antimony (antimonials), which in higher concentrations can cause cardiac, liver and kidney damage. Use of niosomes in tests conducted showed that it was possible to administer higher levels of the drug without the triggering of the side effects, and thus allowed greater efficacy in treatment.

Delivery of Peptide Drugs:

Oral peptide drug delivery has long been faced with a challenge of bypassing the enzymes which would breakdown the peptide. Use of niosomes to successfully protect the peptides from gastrointestinal peptide breakdown is being investigated. In an invitro study conducted by Yoshida et al, oral delivery of a vasopressin derivative entrapped in niosomes showed that entrapment of the drug significantly increased the stability of the peptide.

Use in Studying Immune Response:

Due to their immunological selectivity, low toxicity and greater stability; niosomes are being used to study the nature of the immune response provoked by antigens.

Niosomes as Carriers for Haemoglobin:

Niosomes can be used as carriers for haemoglobin within the blood. The niosomal vesicle is permeable to oxygen and hence can act as a carrier for haemoglobin in anemic patients.

Transdermal Drug Delivery Systems Utilizing Niosomes:

One of the most useful aspects of niosomes is that they greatly enhance the uptake of drugs through the skin. Transdermal drug delivery utilizing niosomal technology is widely used in cosmetics; in fact, it was one of the first uses of the niosomes. Topical use of niosome entrapped antibiotics to treat acne is done. The penetration of the drugs through the skin is greatly increased as compared to un-entrapped drug. Recently, transdermal vaccines utilizing niosomal technology is also being researched. A study conducted by P. N. Gupta et al has shown that niosomes (along with liposomes and transfersomes) can be utilized for topical immunization using tetanus toxoid. However, the current technology in niosomes allows only a weak immune response, and thus more research needs to be done in this field.

Other Applications:

a) Sustained Release:

Azmin *et al* suggested the role of liver as a depot for methotrexate after niosomes are taken up by the liver cells. Sustained release action of niosomes can be applied to drugs with low therapeutic index and low water solubility since those could be maintained in the circulation via niosomal encapsulation.

b) Localized Drug Action:

Drug delivery through niosomes is one of the approaches to achieve localized drug action, since their size and low penetrability through epithelium and connective tissue keeps the drug localized at the site of administration. Localized drug action results in enhancement of efficacy of potency of the drug and at the same time reduces its systemic toxic effects e.g. Antimonials encapsulated within niosomes are taken up by mononuclear cells resulting in localization of drug, increase in potency and hence decrease both in dose and toxicity. The evolution of niosomal drug delivery technology is still at an infancy stage, but this type of drug delivery system has shown promise in cancer chemotherapy and anti-leishmanial therapy.

1.14. Disease overview [17]

HIV infection is caused by retroviruses. Retroviruses have an RNA genome and the unique property of transcribing a DNA of the RNA genome following penetration of the host cells. The DNA is then used as a template to transcribe new RNA Viral copies – thus the term retrovirus. Retroviruses generally evade host immune responses, and cause persistent infection in several species. HIV has a core consisting of the RNA genome and core protein surrounded by an envelope with high lipid content rendering it sensitive to organic solvents.

The unique feature of the virus is that it gains entry to host cells by binding to the CD4 receptor using the viral surface membrane glycoprotein 120. This allows viral attachment and penetration of the host cells. The CD4 receptor is present predominantly on T- helper lymphocytes, which are therefore a major target for the virus. Following penetration of the host cells, the viral RNA is transcribed by the viral enzyme reverse transcriptase into a DNA copy which incorporated into the host cell genomic DNA. This viral DNA may then lie dormant within the cell or undergo replication resulting in transcription of RNA copies and translation to virus proteins resulting in new virus formation and assembly. Viruses then bud from the cell surface. New virus is then available to infect other cells and repeat the process.

2. LITERATURE REVIEW

Kandasamy Ruckmani[18] et al., (2010), prepared and optimized Zidovudine niosomes by altering the proportions of tween, span and cholesterol. The effect of process related variables like sonication time, hydration time, rotation speed of evaporation flask and effect of charge inducing agent was evaluated. Zidovudine niosomes formulated with tween 80 entrapped high amounts of drug and the addition of dicetyl phosphate enhanced the drug release for longer time. The mechanism of release from tween 80 formulation was the Fickian type and obeyed first order release kinetics.

Donatella Paolino[19] et al., (2008), prepared an innovative niosomal system made up of α,ω-hexadecyl bis-(1-aza-18-crown-6) (Bola), Span 80 and cholesterol (2:5:2 molar ratio). It was proposed as a topical delivery system for 5-fluorouracil (5-FU), largely used in the treatment of different forms of skin cancers. Bola-niosomes showed a mean size of 400 nm, which were reduced to 200 nm by a sonication procedure with a polydispersion index value of 0.1. Bolaniosomes showed a loading capacity of 40% with respect to the amount of 5-FU added during the preparation. 5-FU-loaded bola niosomes showed an improvement of the cytotoxic effect with respect to the free drug. Confocal laser scanning microscopy studies were carried out to evaluate both the extent and the time-dependent bola-niosome–cell interaction. Bola-niosomes provided an increase of the drug penetration of 8- and 4-folds with respect to a drug aqueous solution and to a mixture of empty bola-niosomes with a drug aqueous solution.

Mahmoud Mokhtar[20] et al., (2008), prepared niosomes from proniosomal gels. Flurbiprofen niosomes were developed using span 20 (Sp 20), span 40 (Sp 40), span 60 (Sp 60), and span 80 (Sp 80) without and with cholesterol. The influence of different processing and formulation variables such as surfactant chain length, cholesterol content, drug concentration, total lipid concentration, negatively or positively charging lipids, and the pH of the dispersion medium on flurbiprofen EE% was demonstrated. Also, the release of the prepared niosomes in phosphate buffer (pH 7.4) was illustrated. Results indicated that the EE% followed the trend Sp 60 (C18)>Sp 40 (C16)>Sp 20 (C12)>Sp 80 (C18). Cholesterol increased or decreased the EE% depending on either the type of the surfactant or its concentration within the

formulae. Invitro release study shows increase in cholesterol amounts into niosomal formulations could increase the release of flurbiprofen.

Inas A. Darwish[21] et al., (1997) formulated niosomes with 1:1 molar ratio of N-palmitoyl-2 -aminomethyl-15 -crown-5 (PCE) and cholesterol (CHOL) with and without the addition of 10 mol% Solulan C24 (poly-24-oxyethylene cholesteryl ether). A water-soluble fluorescent marker, rhodamine B was encapsulated within these niosomes. Rhodamine B containing PCE/CHOL niosomes of approximately 6.3 mm diameter were visualised by optical microscopy and sonicated PCE/CHOL niosomes of approximately 134 nm in size were visualised by transmission electron microscopy. The release of the fluorescent marker rhodamine B from PCE/CHOL niosomes was slightly increased by the addition of calcium ions but remained unaffected by the addition of sodium ions.

Varaporn Buraphacheep Junyaprasert[22] et al., (2008) investigated the influence of different types of membrane additives including negative charge (dicetylphosphate, DCP), positive charge (stearylamine, STR) and non-ionic molecule (cholesteryl poly-24-oxyethylene ether, SC24) on the physicochemical properties of drug-free and drug-loaded niosomes. Salicylic acid having different proportions of ionized and unionized species at different pH was selected as a model drug. The results shows addition of the membrane additives changed the zeta potential depending on the type of the additives.The particle sizes of all developed niosomes were between 217 to 360 nm. The entrapment efficiency (%E.E.) of all salicylic acid niosomes at pH 3 was higher than that of niosomes at pH 5. After 3 months of storage at 4°C, the particle size of the niosomes remained in the nanosize range except for DCP salicylic acid niosomes at pH 3 whose size increased due to instability of DCP at low pH. All niosomes showed no leakage of the salicylic acid after 3 months of storage indicating the good stability.

P. Arunothayanun[23] et al., (2000) prepared two niosomal systems based on a hexadecyl diglycerol ether (C16G2), cholesterol and poly-24-oxyethylene cholesteryl ether (Solulan C24) in the molar ratios 91:0:9 and 49:49:2 were prepared. In this investigation, the effects of processing variables, particularly temperature and sonication, on the physical characteristics and phase transitional behaviour of two

niosomal systems based on a hexadecyl diglycerol ether (C16G2) have been studied. Entrapment of 5(6)-carboxyfluorescein, particle size and morphology were examined. the polyhedral systems containing C16G2 and Solulan C24 were found to have a larger particle size and higher carboxyfluorescein entrapment efficiency.

Prabagar Balakrishnan[24] et al., (2009) reported minoxidil niosomes for enhanced skin delivery and niosomes efficiency, particle size, zeta suggest that these niosomal formulations could constitute a promising approach for topical delivery of Minoxidil in hair loss treatment as a possible potential and stability. Skin permeation studies were performed using static vertical diffusion franz cells hairless mouse skin and the results approach to improve the low skin penetration and bioavailability shown by conventional topical vehicle for minoxidil niosomes using characteristics thin film hydration method. The result suggests that these niosomal formulations have greater potential for drug cutaneous targeting and could be used as a feasible cargo carrier for the topical delivery of minoxidil in skin diseases such as hair loss.

Aranya Manosroi[25] et al., (2008), formulated niosome encapsulated D- (+) -glucose by using a novel supercritical carbon dioxide fluid (scCO2) technique. Niosomes were composed of Tween61/cholesterol at 1:0, 3:1, 1:1, 1:3 and 0:1 molar ratios and entrapped with D -(+) glucose by the scCO2 method without and with ethanol, and the conventional chloroform film method with sonication. Tween61/cholesterol at 1:1 molar ratio niosomes prepared by all methods exhibited the best physical stability. Niosomes by the scCO2 method with 10% (w/w) ethanol gave higher entrapment efficiency (12.22±0.26%) than those by the conventional chloroform film method with sonication (10.85±0.24%) and the scCO2 method without ethanol (8.40±1.60%).

Ahmed S. Guinedi[26] et al., (2005), reported as niosome is a novel approach to improve the low corneal penetration and bioavailability characteristics shown by conventional ophthalmic vehicles. Niosomes formed from Span 40 or Span 60 and cholesterol in the molar ratios of 7:4, 7:6 and 7:7 were prepared using reverse-phase evaporation and thin film hydration methods. The prepared systems were characterized for entrapment efficiency, size, shape and in vitro drug release. Stability studies were carried out to investigate the leaching of drug from niosomes during storage. The results showed that the type of surfactant, cholesterol content and the

method of preparation altered the entrapment efficiency and drug release rate from niosomes. Higher entrapment efficiency was obtained with multilamellar niosomes prepared from Span 60 and cholesterol in a 7:6 molar ratio. Niosomal formulations have shown a fairly high retention of acetazolamide inside the vesicles (approximately 75%) at a refrigerated temperature up to a period of 3 months.

Parinya arunothayanun[27] et al., (1999), formulated Luteinizing hormone releasing hormone (LHRH) loaded niosomes by various methods such as direct hydration at pH 7.4., direct hydration at pH 3.0, dehydration-rehydration of vesicles (DRV), reversed-phase evaporation of vesicles, remote loading by (NH4)2SO4 gradient., and remote loading by pH gradient. In this higher level of entrapment is obtained in niosomes formulated by active loading methods. [i.e. $(NH4)_2SO4$ gradient and pH gradient].

Sanyog Jain[28] et al., (2005) developed mannosylated niosomes as a topical vaccine delivery carrier and adjuvant for the induction of both humoral and cellular immunity. Bovine serum albumin (BSA) - loaded niosomes composed of sorbitan monostearate/sorbitan trioleate (Span 60/Span 85), cholesterol and stearylamine as constitutive lipids were prepared by the reverse-phase evaporation method. The niosomes were coated with a modified polysaccharide O-palmitoyl mannan (OPM) to target them to Langerhan's cells, the major antigen presenting cells found in abundance beneath the stratum corneum. Prepared niosomes were characterized in-vitro for their size, shape, entrapment efficiency and ligand binding specificity. The serum IgG levels were significantly higher for the mannosylated niosomes as compared with plain uncoated niosomes.

Zerrin sezgin bayindir[29] et al., (2010), prepared Paclitaxel (PCT) loaded niosomes from the self-assembly of nonionic amphiphiles in aqueous media using different surfactants (Tween 20, 60, Span 20, 40, 60, Brij 76, 78, 72) by film hydration method. PCT was successfully entrapped in all of the formulations with encapsulation efficiencies ranging between 12.1-1.36% and 96.6-0.482%. PCT released from niosomes by a diffusion controlled mechanism. The slow release observed from these formulations might be beneficial for reducing the toxic side effects of PCT.

Depending on the addition of the negatively charged dicetyl phosphate to the formulations negative zeta potential values were obtained.

Deepika Aggarwal[30] et al., (2004), formulated niosomes to enhance the bioavailability of acetazolamide by the topical route and to improve the corneal permeability of the drug, niosomes of acetazolamide were prepared (employing span 60 and cholesterol) by different methods. Niosomes were also prepared in the presence of dicetyl phosphate and stearylamine to obtain negatively and positively charged vesicles, respectively. It was found that the reverse - phase evaporation method gave the maximum drug entrapment efficiency (43.75%) as compared with ether injection (39.62%) and film hydration (31.43%) techniques. Drug entrapment efficiency varied with the charge and the percent entrapment efficiency for the REV method was 43.75, 51.23 and 36.26%for neutral, positively charged and negatively charged niosomes, respectively.

E. O. Confalonieri[31] et al., (2009) studied pharmacokinetic parameters of flurbiprofen (FBP) after intravenous (i.v.) administration (0.5 mg⁄kg) of niosomal and nonniosomal formulations in dairy cattle. Niosomes of FBP showed a drug loading of 92.0 ± 0.7% and the intravenous administration of the FBP niosomes to dairy cattle did not produce any immunological reaction associated to niosomal components. Niosomal FBP was slowly eliminated from plasma and mean residual time (MRT) and AUC_{0-t} and $t_{½}$ values were significantly higher than those for non niosomal FBP formulations. The results presented in this study indicate that the long circulation of FBP niosomes offers a potential application for improving the pharmacokinetic parameters of short half-life drugs for clinical use. Niosomes offer new promising perspectives of drug delivery modules in bovine therapeutics.

Roopa Karki[32] et al., (2008), studied niosomes as alternates to liposomes. An increasing number of non-ionic surfactant has been found to form vesicles, capable of entrapping hydrophilic and hydrophobic molecules. Isoniazid encapsulated as formulation using ethanol injection method. A different ratio of cholesterol was used. The formulated systems were characterised for *in vitro* by size distribution analysis, drug entrapment efficiency and drug release profiles. *In vivo* drug disposition was evaluated in normal, healthy albino rats for niosomal formulation. The size range

2.28±0.008 (plain Span 60), 2.311±0.009 (Span60: Cholesterol, 40:50), 2.15±0.002 (Span60: Cholesterol 50:50). The entrapment release 74.12% (Plain Span 60), 80.23% (Span60: Cholesterol, 40:50), 76.26% (Span60: Cholesterol 50:50). *In vitro release* pattern indicated that about total drug content were released within 48 h. The niosomal drug delivery system has lesser toxicity than free drug.

Minghuang Hong[33] et al., (2009), repoted that the possibility of combination of the stealth action by polyethylene glycol cyanoacrylate-co-hexadecyl cyanoacrylate (PEG-PHDCA) modified niosomes and active targeting function of transferrin (Tf) by transferrin receptor-mediated endocytosis to promote drug delivery to solid tumor following intravenous administration with hydroxyl camptothecin (HCPT) as model drug. The HCPT loaded PEG-niosomes (PEG-NS) were prepared by thin-film hydration and ultrasound method; the periodate-oxidated Tf was coupled to terminal amino group of PEG to produce the active targeting vesicles with average diameters of 116 nm. The uptake of Tf-PEG-NS into KB cells was concentration and time dependent, which could be inhibited by low temperature and free Tf, indicating that the endocytosis process was energy-driven and receptor specific. Compared with HCPT injection, non-stealth niosomes and PEG-NS, Tf-PEG-NS demonstrated the strongest cytotoxicity to three carcinomatous cell lines (KB, K562 and S180 cells), the greatest intracellular uptake especially in nuclei, the highest tumor concentration and largest area under the intratumoral hydroxycamptothecin concentration curve, as well as the most powerful anti-tumor activity with the inhibition rate of 71% against S180 tumor in mice. The results showed that the transferring modified PEGylated niosomes could be one of the promising solutions to the delivery of anti-tumor drugs to tumor.

Karthikeyan. D[34] et al., (2009), prepared diclofenac sodium loaded niosomes by lipid film hydration method using span 60 and cholesterol with various molar ratios and characterized for *invivo* drug release study The molar ratio of 100:60 showed higher entrapment of drug and released 79.34 % ± 1.04 at10th h was used for the *invivo* drug release study. The molar ratio of 100:60 showed higher entrapment of drug and released 79.34 % ± 1.04 at 10th h was used for the *invivo* drug release study. The ocular irritation test of niosome containing diclofenac sodium was found to be safe which was confirmed by histopathological study.

R.A. Raja naresh [35] et al., (1996), prepared niosomes for targeting the delivery of anticancer drugs to the tumor site more quantitatively. In this direction attempts have been made to activate and exploit macrophages in delivering niosomal and thermosensitive niosomal bleomycin more quantitatively to tumor site using niosome encapsulated immunomodulators muramyl dipeptide and tuftsin. Niosomal bleomycin and thermosensitive niosomal bleomycin were prepared by lipid layer hydration method. The antitumor efficacy was assessed using two tumor models viz. Sarcoma-180 and ehrlich ascites using Balb/C mice. Tumor distribution profiles of bleomycin before and after macrophage activation were studied in tumor bearing mice. The mean survival time of ehrlich ascites infected mice increased significantly after macrophage activation. Accumulation of higher bleomycin levels after macrophage activation exerted increased antitumor effect. The present study suggested that a more quantitative delivery of bleomycin encapsulated in niosomes, to the tumor site is possible after macrophage activation.

Aliasgar[36] et al., (2002), selected lipid film hydration technique to prepare niosomes containing nimesulide. The encapsulation of drug in niosome modified the bio distribution of drug in rats , which has shown higher and sustained plasma drug level profile compared to free drug solution. Prepared niosomes were analyzed for percent drug entrapment. The percent drug entrapment decreased (10.05% ± 0.008%) with decrease in HLB from 8.6 to 1.8 (Span 85).

Pandya Hima[37] et al., (2011), prepared and evaluated Trihexyphenidyl encapsulated niosomes by the thin film hydration method by using cholesterol and span 60 as a surfactant in different ratios. The formulation T2 (cholesterol:span40, 200:200) was showing the satisfactory particle size 4.6 ± 0.5 µm, entrapment efficiency 76.35 ± 0.9% and in vitro drug release 78.8% for the extended period of time. So from the result we can conclude that the niosomes may be apromising carrier for Trihexyphenidyl and other drugs, especially due to their simple production and facile up.

Shamsheer Ahmad S[38] et al., (2011), formulated Lisinopril proniosomal gels by using Lecithin, Cholesterol as encapsulating agents, Surfactant, Span and permeation enhancers. The FTIR studies conducted revealed that there no interaction between

Lisinopril and excipients which. The physical characterization of proniosomal gels was found to be within the acceptable limits. It was observed that the gel formulations showed good spreadability and viscosity. Determination of vesicle size was found to be 20.10-26.23µm. The proniosomes showed spherical and homogenous structure in optical microscopy. All formulations showed zero order drug release by diffusion mechanism. The stability studies showed that proniosomal gels were stable at 4 to 8°C and 25±2°C. The above results indicated that the proniosomal gels of could be formulated for controlled release of Lisinopril.

M.A. Shatalebi[39] et al., (2010), formulated the N-acetyl glucosamine (NAG) niosomes and investigated its flux across excised rat skin using Franz diffusion cells. The drug assay was performed by a novel and specific high performance liquid chromatography method. Niosomal vesicles were further characterized by optical and scanning electron microscopy and particle size analysis. Niosomes prepared with Span 40 produced a drug encapsulation of about 50%. The vesicle size was markedly dependent on the composition of the niosome formulations and was in range of 500-4500 nm (Span 80 < Span 60 < Span 40 niosomes). Span 40- niosomes provided a higher NAG flux across the skin than Span 60- and Span 80-nisomes. All formulations significantly improved the extent of drug assessed to be localized in the skin ($P< 0.05$), as compared to NAG hydroalcoholic (HA) solution. Our study demonstrated the potential of niosomes for improved NAG localization in the skin, as needed in hyperpigmentation disorders.

Ibrahim A. Alsarra[40] (2008), prepared Piroxicam-loaded niosomes and characterized for surface morphology, entrapment efficiency and in vitro permeation across excised rat skin from various proniosome gel formulations using Franz diffusion cells. Proniosomes prepared with Span 60 provided a higher piroxicam flux across the skin than did those prepared with Tween 80. Niosomes prepared using Span 60 showed a higher release rate than those prepared using non-ionic surfactants, Span 20 and Span 80, while those prepared from Tween showed higher release rate than formula prepared with Span. This indicates that lipophilicity and hydrophilicity of surfactant has a main role in release rates of piroxicam. Particle size of piroxicam niosomal vesicles formed by proniosome was determined by scanning electron microscopy. The encapsulation efficiency was evaluated by a specific high performance liquid

chromatography method. Niosomes formed from using Spans and Tweens exhibited very high encapsulation efficiency. The results are very encouraging and suggest that niosomes can act as promising carriers offering an alternative approach for transdermal delivery of piroxicam.

N.Pavala rani[41] et al., (2010), prepared niosomes of rifampicin and gatifloxacin by lipid hydration technique using rotary flash evaporator. The prepared rifampicin and gatifloxacin niosomes showed a vesicle size in the range of 100-300nm, the entrapment efficiency were 73% and 70% respectively. The *invitro* release study showed that 98.98% and 97.74% of release of rifampicin and gatifloxacin niosomes respectively. The bactericidal activities of the niosomal formulation were studied by BACTEC radiometric method using the resistant strains (RF 8554) and sensitive strains (H37Rv) of *Mycobacterium tuberculosis* which showed greater inhibition and reduced growth index.

Arora Rajnish[42] et al., (2010), formulated vesicles containing Ketoconazole using different non-ionic surfactants by Thin film hydration technique. The results confirmed that the relative slow release pattern of the entrapped drug from niosomes.

Anita R. Desai[43] et al., (2010), prepared niosomes containing α-lipoic acid. by reverse phase evaporation method using span and tween (20 and/or 60). The preparations were characterize with respect to size reduction, entrapment efficiency, *in-vitro* drug release profile and stability under specific conditions. The diameter of niosomes ranges from 1-3μm with spherical/ oval in shape. Higher cumulative release was observed with 76.202 % for span-20, 69.72% for span-60, 81.78 % for tween-20 and 84.06 % for tween-60 respectively. Stability studies proved that optimum storage condition for niosomes was found to be 4°c.

Pandey Shivanand[44] et al., (2010), used Thin film Hydration Technique to prepare Cefpodoxime proxetil encapsulated niosomes with various surfactants like Span 20, 40 and 80. The incompatibility between the drug and excipients shows that span is suitable for cefpodoxime proxetil to prepare niosomes. The *in vitro* release studies and kinetic study declared the release is zero order and in controlled manner, so there will be not chances of dose dumping during usage.

Ismail A. Attia [45] et al., (2007), improved poor and variable oral bioavailability of Acyclovir using niosmal formulation. The nonionic surfactant vesicles were prepared by the conventional thin film hydration method. The lipid mixture consisted of cholesterol, span 60, and dicetyl phosphate in the molar ratio of 65:60:5, respectively. The percentage entrapment was 11% of acyclovir used in the hydration process. *In vitro* drug release profile was found to follow Higuchi's equation for free and niosomal drug. The niosomal formulation exhibited significantly retarded release compared with free drug. The in vivo study revealed that the niosomal dispersion significantly improved the oral bioavailability of acyclovir in rabbits after a single oral dose of 40 mg kg−1. The average relative bioavailability of the drug from the niosomal dispersion in relation to the free solution was 2.55 indicating more than 2-fold increase in drug bioavailability..In conclusion, the niosomal formulation could be a promising delivery system for acyclovir with improved oral bioavailability and prolonged drug release profiles.

Giulio Caracciolo[46] et al., (2008), prepared niosomal vesicles (Niosomes) using binary mixture of polysorbate 20 (Tween 20) and Cholesterol in aqueous solution. And investigated by means of synchrotron small angle X-ray scattering (SAXS). Solid-supported niosomal membranes at full hydration exhibit the same structural properties, as determined by in situ energy dispersive X-ray diffraction (EDXD), than their counterpart in solution. Both Niosomes and solid-supported niosomal membranes are made of highly swollen bilayers rich in Tween 20 coexisting with Cholesterol crystallites. EDXD patterns from oriented samples suggest that at least some Cholesterol crystals are aligned along the normal to the solid support.

Elsie Oommen[47] et al., (1999), prepared Methotrexate- β-Cyclodextrin (MTX- βCD) complex entrapped niosomes by lipid layer hydration method. The niosomal entrapment efficiency was higher in the case of MTX - βCD complex (84%) than with the plain drug (67%). Comparison of the drug release profile revealed a relatively slow release pattern of the entrapped drug complex from the vesicles as compared to plain MTX encapsulated niosomes. Better stability on storage was also observed with the niosome entrapped complex. The complex entrapped niosomes produced an improved anticancer activity as evident by enhanced volume doubling time and growth delay.

Ling HU[48] et al., (2008), established a preparation method for the phycoerythrin subunit (PE-sub) liposome. The optimum preparation conditions of the PE-sub liposome were found: a phosphatidylcholine-to-cholesterin ratio of 1:2, a PE-sub-to–lipid ratio of 1:30, 20 ml buffer volume, 10 min sonication time, and an average encapsulation rate of up to 47.2%. The particle size ranged from 80 to 200 nm, and the average particle diameter was 136 nm. At a concentration of 100 μg/ml, the transfection rate of the PE-sub liposome reached 18% at 2 h and 24% at 4 h, and remained steady at 5–6 h. The half lethal dose of PDT on HepG2 was 75 μg/ml, whereas the cell survival rate of HL7702 reached 80% at the same dosage.

Bin Shi[49] et al., (2006), formulated a series of novel niosomes with the amphiphilic copolymer of poly (methoxypolyethyleneglycol cyanoacrylate-co-n-hexadecyl cyanoacrylate) (PEG-PHDCA) acted as surface modification materials and Hydroxycamptothecin (HCPT) was used as a model drug. This work concentrated on the effects of PEG chain length and particle sizes on the niosomes surface properties, in vitro drug release, phagocytic uptake, *in vivo* pharmacokinetics and antitumor activity. Within the range of PEG Mw from 2000 to 10000, the increasing zeta potential (from -16.08 to -5.25 mv) and thicker fixed aqueous layer (3.82 to 5.78 nm) would facilitate the niosomes stealth effects, while the reduced PEG chain density (from 0.53 to 0.17 PEG/nm2) and the quickened speed of drug release would diminish the effects. As a result, the PEG5000-PHDCA niosomes had the least phagocytic uptake, the longest half-life of 11.46 h and the best tumor inhibition rate of 97.1%.

Anil Vangala[50] et al., (2006), improved the stability of Dimethyldioctadecylammonium (DDA) without undermining their potent adjuvanticity by incorporating into non-ionic surfactants, such as 1-monopalmitoyl glycerol (MP), in addition to cholesterol (Chol) and trehalose 6,60-dibehenate (TDB), using lipid hydration method. Differential scanning calorimetry revealed a reduction in the phase transition temperature (Tc) of DDA-based vesicles by 12°C when MP and cholesterol (1:1 molar ratio) were incorporated into the DDA system. Transmission electron microscopy (TEM) revealed the addition of MP to DDA vesicles resulted in the formation of multi-lamellar vesicles. Environmental scanning electron microscopy (ESEM) of MP–Chol–DDA–TDB (16:16:4:0.5-mol) indicated that incorporation of antigen led to increased stability of the vesicles, perhaps as a

result of the antigen embedding within the vesicle bilayers. At 4°C DDA liposomes showed significant vesicle aggregation after 28 days, although addition of MP–Chol or TDB was shown to inhibit this instability. Alternatively, at 25°C only the MP-based systems retained their original size. The presence of MP within the vesicle formulation was also shown to promote a sustained release of antigen in-vitro.

Gupta Naveen[51] et al., (2010), formulated and evaluated Oflaxacin niosomes. In the present investigation nine formulations of Niosomal drug delivery system of ofloxacin with non ionic surfactant, span 60, in various proportions were prepared and evaluated for Morphological characterization, Encapsulation efficiency, *In-vitro* drug release study, Drug release kinetic data analysis, Stability study, Test for significance, Zeta potential analysis, Ocular irritation test, Estimation of minimum inhibitory concentration, *In vivo* study. Niosomes formed from span 60 and cholesterol in the ratio 200:100 (in mol) is a promising approach to improve the bioavailability of Ofloxacin even for an extended period of time which showed good physicochemical properties, good stability and controlled drug release pattern, thereby improving the bioavailability of the drug.

S.Sambhakar[52] et al., (2011), prepared niosomes containing Cefuroxime axetil was by film formation method by Span 40, 60 and 80. It is characterised by SEM for particle size and morphology. The vesicle size was found to be less than 5 μm and its polydispersity index was very low. Entrapment efficiency was found as Span 60 > Span 40 > Span 80. The in-vitro-release study indicated the controlled release profile of niosomes. Absorption study by everted-sac method showed that maximum absorption was found in case of niosomes containing bile salts and Span 60 followed by niosomes containing Span 60 only and then Span 40. Stability study indicated that on incorporation of bile salt upto 7.5 μM in the vesicle as integral component, the stability was maximum on exposure to 20 μM bile salt, whereas we omit incorporation of bile salt in vesicles, it showed least stability.

K. Srikanth[53] et al., (2010), prepared Meloxicam entrapped niosomes prepared by Thin film hydration technique using Nonionic surfactants (Span-80, Span-60, Tween-80 and Tween-60), Cholesterol and drug in different ratios. The prepared niosomes appeared as round in shape and size range was found to be 1.54 – 2.64μm. Among all

formulations FS804 (surfactanant: drug: cholesterol, 2:1:1) shows high entrapment efficiency and consistent drug release pattern.The *iv-vivo* studies were demonstrated that niosomal gel was (FS804) shown better pharmacological activity than the conventional meloxicam gel. Based on the results it was concluded that niosomal preparation offers more advantages than the conventional preparation.

Rishu Kakkar[54] et al., (2011), prepared niosomes containing Valsartan and characterised for encapsulation efficiency, shape, size and in vitro drug release and stability study. The results showed that valsartan in all the formulations was successfully entrapped and a substantial change in release rate and an alteration in the encapsulation efficiency of valsartan from proniosomes were observed upon varying the type of surfactant and cholesterol content. The encapsulation efficiency of proniosomes prepared with Span 60 was superior to that prepared with Span 40. A preparation with 9:2:9 ratio of Span 60, cholesterol and lecithin gave maximum encapsulation efficiency (71.50%) and release results (Q24h= 75%) as compared to other compositions. Proniosomal formulations showed fairly high retention of valsartan inside the vesicles at refrigerated temperature (4-8°C) up to 1 month.

Mohammed Shafik El-Ridy[55] et al., (2011), Prepared Pyrazinamide (PZA) niosomes using different molar ratios of Span 60 and Span 85, with cholesterol (CH) i.e. Span: CH (1:1) and (4:2). Dicetyl phosphate and stearyl amine were used in preparation of negative and positively charged niosomes, respectively. Free PZA was separated by cooling centrifugation and estimated spectrophotometrically at 268.4 nm. Niosomes were characterized by electron microscopy and differential scanning calorimetry. The highest percentage PZA entrapped was obtained using Span 60 and the molar ratio (4:2:1) negatively charged niosomes. This was followed by the neutral PZA neutral (4:2) Span 60 niosomes.

Mohamed S. El-Ridy[56] et al., (2011), formulated silymarin niosomes with enhanced activity and limited side effects. Silymarin loaded niosomes were prepared using different non-ionic surfactants (NIS), cholesterol (Ch) and different charge inducing agents (CIA) in molar ratios (1:1:0.1) and (2:1:0.25). The effect of components molar ratio and effect of surface charges on the percentage drug encapsulated were

investigated. Characterization of prepared niosomes was performed via transmission electron microscopy (TEM), differential scanning calorimetry (DSC), particle size analysis and also investigation of the *in vitro* release profiles. Selected silymarin niosomal formulations were evaluated for their hepatoprotective activity against carbon tetrachloride (CCl4) induced oxidative stress in albino rats. Biochemical parameters like serum glutamate oxaloacetic transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and serum alkaline phosphatase (SALP) were used to measure the degree of liver protection. Silymarin niosomal formulations produced a significant decrease in both transaminase levels as well as in SALP level in comparison with administered silymarin suspension.

Raj K. Keservani[57] et al., (2011), determined the effect of different process variables on the preparation of baclofen vesicles (Niosomes) and results that vesicle size increases on entrapment of drug. Effect of amount and type of surfactant shows niosome size directly proportional to the HLB value of the surfactants. On increasing the cholesterol content of bilayer shows decrease rate of release of encapsulated material. Methods of preparation can also affect and produce different sized niosomes when prepared by different methods. Osmotic effect of niosomes in hypertonic media produces Shrinked type of niosomes whereas in hypotonic media niosomes are Swells or burst type. 6 ml hydration medium and 4 h hydration time for maximum % drug entrapment that is 87.90±0.18 % and 94.81±0.56 % respectively.

C. P. Jain[58] et al., (1995), prepared niosomes (non-ionic, surfactant-based vesicles) containing rifampicin of 8- 15 μm in diameter using Span-85 and cholesterol in various molar fractions. The process variables that could affect the physical characteristics of niosomes and *in vitro* release of the drug from the niosomes were studied and optimized. *In vivo* distribution studies of the prepared niosomes found that 65% of the drug could be localized in the lungs by controlling the niosome size.

Waraporn Suwakul[59] et al., (2006), formulated and evaluated Propylthiouracil (PTU) niosomes. The results revealed that niosomes readily formed from various compositions of nonionic surfactant and cholesterol, with or without a stabilizer. Entrapment of PTU in niosomes depended on bilayer composition. The release of PTU from all niosomal formulations studied was retarded and followed the first-order

kinetics. Degree of slow release had a negative correlation with drug entrapment. The release rate also depended on the physical state of the bilayer. The results of this study indicate that PTU niosomes were able to control the release of PTU and might be of value to develop further into topical formulations.

Meiying Ning[59] et al., (2005), prepared and optimized the Insulin loaded niosomes. The results showed optimized niosomes prepared in this study had niosomal entrapment efficiency 26.68±1.41% for Span 40 and 28.82±1.35% for Span 60, respectively. The particle sizes of Span 40 niosomes and Span 60 niosomes were 242.5±20.5nm and 259.7±33.8 nm, respectively. Compared with subcutaneous administration of insulin solution, the relative pharmacological bioavailability and the relative bioavailability of insulin-Span 60 vesicles group were 8.43% and 9.61%, and insulin-Span 40 niosomes were 9.11% and 10.03% ($p > 0.05$). Span 60 and Span 40 niosomes were both higher than blank Span 40, Span 60 vesicles, and free insulin physical mixture groups ($p < 0.05$). The results indicates insulin-Span 60, Span 40 niosomes had an enhancing effect on vaginal delivery of insulin.

Ajay B. Solanki[60] et al., (2010), prepared Aceclofenac encapsulated niosomes by thin film hydration technique. A 3^2 factorial design was utilized to study the effect of the molar ratio of drug to lipid ($X1$) and volume of hydration medium ($X2$) on percentage drug entrapment (PDE) and vesicle size. Selected batches of niosomes were incorporated in to carbopol gel matrix to prepare the niosomal gel formulations, which were evaluated for *in-vitro* release, skin permeation and *in vivo* studies. It was evident from the derived polynomial equations and constructed contour plot, a decrease in the level of $X1$ and an increase in the $X2$ lead to an increase in PDE and decrease in vesicle size. The polynomial equations and contour plot predicted the levels of independent variables $X1$ and $X2$ (0.19 and 0.46 respectively), for maximized response of PDE with constraints on vesicle size. Each of the prepared niosomal gel formulations significantly improved ($P<0.05$) cumulative amount of drug permeated, steady state transdermal flux and increase in paw thickness. Among the niosomal gel formulations, NA6 (prepared at high level of $X1$ and medium level of $X2$) showed best permeation and effectiveness may be due to efficient hydration of the film and more total amount of drug entrapped.

Chawda Himmat Singh[61] et al., (2011), designed suitable niosome-encapsulated drug delivery for nimesulide and evaluated the vesicle size, encapsulation efficiency, in vitro release and physical stability of the system. Non-ionic surfactants used were span 20, 40, 60 and cholesterol was used in different molar ratios. The niosomes prepared by lipid film hydration method were multilamellar vesicles (MLVS) and niosomes prepared by ether injection technique were unilamellar vesicles (ULVS) or oligolamellar vesicles. The higher entrapment efficiency was observed with MLVS prepared from span 60 and cholesterol in an 80:70 molar ratio. The release pattern shown by these formulations were zero order & Higuchi diffusion controlled mechanism. The physical stability study show that niosomal preparation stored at refrigerated temperature for 60 days show maximum drug retained for all the formulation compare to room temperature and elevated temperature conditions.

Muhammad Naveed Yasin[62] et al., (2012), prepared Chloramphenicol niosomes using two different ratios of cholesterol, drug and surfactant, termed as EIN-1 (drug: surfactant: surfactant, 1:1:1), EIN-2 (1:1:2) by ether injection method and their entrapment efficiency, particle size. The *in vitro* drug release pattern was observed for ten hours. The EIN-2 showed 90% entrapment and released 81% of entrapped drug after 10 hours. Zeta potential & viscosity were determined and in-vivo comparison was made with Chloramphenicol eye drops where it exhibited C max of 15µg/ml. Stability studies were done to determine shelf life. MIC of selected strain of *S. aureus* was also determined. EIN 2 niosomal suspension was In-vitro studies are encouraging as niosomes released about 75% of total entrapped drug by EIN-1 and 81% of total entrapped drug by EIN 2.compared with Chloramphenicol eye drops in experimental conjunctivitis in albino rabbits.

P.U.Mohamed Firthouse[63] et al., (2011), prepared Miconazole niosomes by varying the cholesterol and surfactant ratios as 1:0.5, 1:1, and 1:1.5. Each formulation was evaluated for percentage of drug entrapment and for their cumulative drug release. Formulation with 1:1 CHOL: SA ratio, the concentration of SA was increased and it has shown 92.10 % drug release in 24 hours. The release showing required amount of drug release per day as well as extended for the required day is the optimized formulation. Hence, B (1:1) formulation is the optimized one.

A. Abdul Hasan Sathali[64] et al., (2010), formulated niosomes of Terbinafine hydrochloride by thin film hydration method using different ratios of non ionic surfactant (tween 20, 40, 60, and 80) and cholesterol with constant drug concentration. The prepared formulations were evaluated for its vesicle size (by AFM), entrapment efficiency (by dialysis method) *in vitro* release studies and antifungal activities. Increase in surfactant concentration, increased the entrapment efficiency (up to 84.92%) and the formulation with surfactant cholesterol ratio 2:1 in each group of surfactant showed good entrapment. Niosomal preparation were tested for *in vitro* antifungal activity using the strain *Aspergillus niger* and compared with pure drug solution (as standard). The best formulation with maximum zone of inhibition and sustained release of drug (tween 40 nisomes) incorporated into gel bases and evaluated. The studies revealed that gel containing total niosomes possess maximum zone of inhibition values (12mm) initially followed by sustained release (12mm-16mm) comparing to gel containing drug entrapped niosomes, gel containing pure drug and marketed preparation.

Anupriya kapoor[65] et al., (2011), formulated Acyclovir loaded niosomes formed using sorbitan esters (Span 20, 40, 60, and 80) and cholesterol in different molar ratio. Niosomes were formed using Reverse phase evaporation method. The so formed niosomes were characterized for their *in vitro* drug release efficiency. The results indicated that more sustained release pattern can be obtained by incorporating the drug in niosomes formed with Span60.

5. MATERIALS AND METHODS

5.1. MATERIALS

Table:2 Materials used

S.NO	Materials	source
1.	Zidovudine	Bafna pharmaceuticals,Mumbai
2.	Potassium di hydrogen phosphate	S.D. Fine Chem Ltd, Boisar
3.	Disodium hydrogen phosphate	S.D. Fine Chem Ltd, Boisar
4.	Sodium chloride	S.D. Fine Chem Ltd, Boisar
5.	Sorbitan mono laurate (span 20)	S.D. Fine Chem Ltd, Boisar
6.	Polysorbate 20 (tween 20)	S.D. Fine Chem Ltd, Boisar
7.	Cholesterol	Qualigens Chem Ltd, Boisar
8.	Chloroform	S.D. Fine Chem Ltd, Boisar
9.	Methanol	S.D. Fine Chem Ltd, Boisar
10.	Dicetyl phosphate	S.D. Fine Chem Ltd, Boisar
11.	Triton X – 100	S.D. Fine Chem Ltd, Boisar

5.2. INSTRUMENTS

Table:3 Instruments used

S.NO	Equipments	Company
1.	Rotary flash evaporator	Equitron, Mumbai.
2.	Probe sonicator	Bandelin, Germany
3.	UV- Visible spectrophotometer	Shimadzu corporation, Japan
4.	Stability chamber (120 litres)	Osworld, Mumbai
5.	Single pan electronic balance	Shimadzu corporation
6.	Magnetic stirrer	Remi motor Ltd, Mumbai
7.	pH meter	ELICO, Pvt, Ltd.Chennai.
8.	Autoclave	Dalal, Chennai.
9.	Laminar air flow bed	Klenzoids, Mumbai
10.	Hot air oven	Biochem, Mumbai.
11.	IR Spectrophotometer	Perkin Elmer, Germany
12.	Cooling centrifuge	Remi motor Ltd. Mumbai.

5.3. DRUG PROFILE[66, 67]

ZIDOVUDINE

Synonym

Azidothymidine

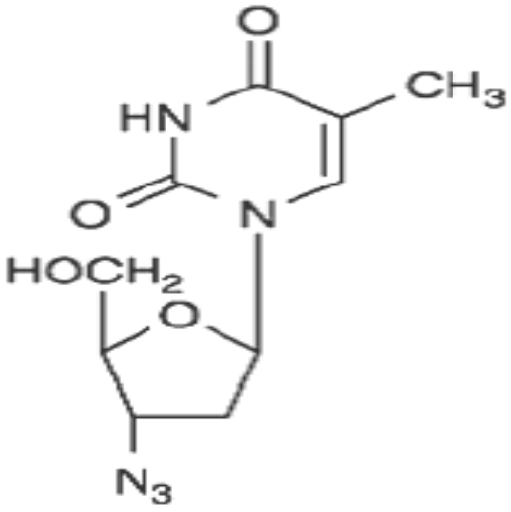
Chemical name

1-(3-azido-2,3-deoxy-β-D-furanosyl)-5-methyl pyrimidine-2,4(1H,3H)-dione.

Therapeutic category

Anti retroviral

Structure



Molecular formula

$C_{10}H_{13}N_5O_4$

Melting range

122°C to125°C

Storage

Protected from light.

Description

White or almost white powder.

Odourless and crystalline solid.

Solubility

Sparingly soluble in water. Soluble in ethanol.

Dose [68]

Dose by mouth 500 – 600 mg in 2 – 3 divided doses.

Child over three months 360 – 480 mg/m^2 daily in 3 – 4 divided doses.

By intravenous over1 hr, 1-2 mg/kg.

Mechanism of action

Zidovudine is a nucleoside reverse transcriptase inhibitor which is phosporylated by host cell enzymes to give 5´triphosphate derivative. The moiety competes with equivalent host cellular triphosphate substrate for proviral DNA synthesis by viral reverse transcriptase (viral RNA dependent DNA polymerase), eventually incorporation of the 5´triphosphate moiety into the growing viral DNA chain results in chain termination.

Pharmacokinetics

It is generally administered orally a twice daily. But can also given by intravenous infusion. The bioavailablity is 60-80%, and the peak plasma concentration at 30 min. Half life of zidovudine is 1 hour and the intracellular half life of the active triphosphate is 3 hours. The concentration of cerebrospinal fluid is 65% of the plasma level. Most of the drug is metabolised to the inactive glucuronides in the liver, and 20% 0f active form being excreted in urine.

Side effects

Blood disorders – anaemia, neutropenia, and thrombocytopenia.

GI disturbances – nausea, vomiting, abdominal pain and diarrhoea.

CNS effects – headache, insomnia, and dizziness.

Therapeutic indications

It can prolong life in HIV infected individual and diminish HIV associated Dementia.

5.4. EXCIPIENTS LITERATURE [69]

Cholesterol

Synonym

Cholesterin

Chemical formula

Cholest – 5 – en – 3 β - ol

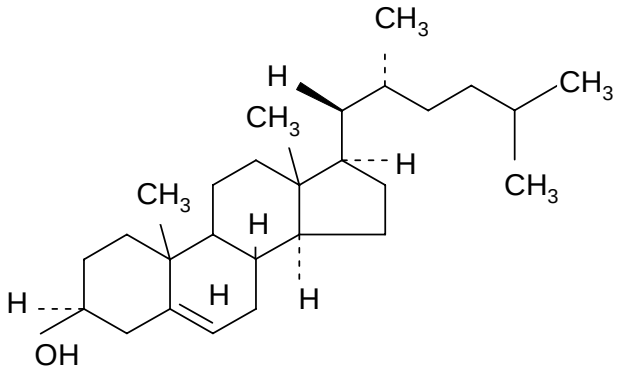
Empirical formula

C_{27} $H_{46}O$

Molecular weight

386.67

Structural formula



Functional category

Emulsifying agent, Emollient

Application in pharmaceutical formulations

- It is used as emulsifying agent in cosmetic and topical pharmaceutical formulation.

Description

- White or faintly yellow colour.
- Almost odourless.
- Pearly leaflets, needles, powder or granules.

Typical properties

Density : 1.052 g/cm^3

Boiling point : 360^{o}C

Melting point : 147 – 150^{o}C

Specific rotation : -34^o to -38^o

Solubility

- Soluble in acetone, vegetable oil.
- Soluble in organic solvents like benzene, methanol, hexane, chloroform, ethanol, ether.

Stability and storage conditions

Cholesterol is stable and should be stored in a well closed container, protected from light.

Incompatibilities

Precipitated by digitonin.

Sorbitan mono laurate

Synonym:

Span 20

Chemical name:

Sorbitan monododecanoate.

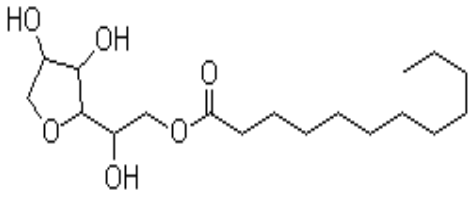
Empirical Formula:

$C_{15}H_{34}O_6$

Molecular weight:

346

Structural Formula



Functional Category:

Nonionic surfactant, emulsifying agent, solubilising agent, wetting agent.

Application in Pharmaceutical Formulation:

- They are widely used in pharmaceutical formulation as emulsifying agent in the preparation of creams, emulsions and ointments for topical application.
- They are widely used in cosmetics, food products, pharmaceutical formulations as lipophilic nonionic surfactant.

Description:

- Yellow viscous liquid
- Distinctive odour and taste.

Typical properties:

Density - 1.01 gm/cm^3

Surface tension - 28(mN/M)

HLB value - 8.6

Solubility:

- Soluble or dispersible in oils.
- They are also soluble in most of organic solvents.

Stability and Storage:

Stored in a well closed container in a cool dry place.

Polysorbate 20

Synonym

Tween 20

Chemical name

Polyoxyethylene 20 sorbitan monolaurate.

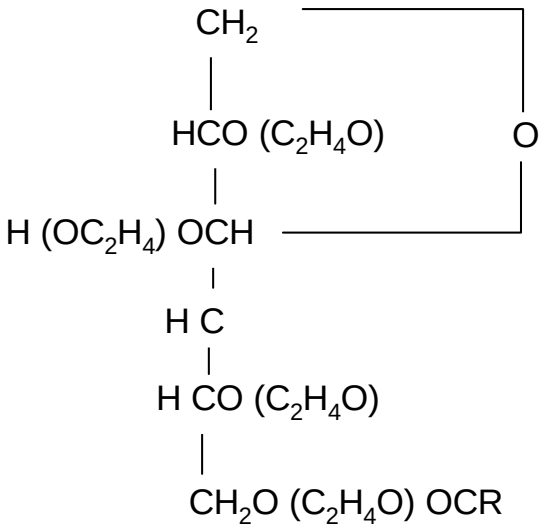
Empirical formula

$C_{58}\ H_{114}O_{26}$

Molecular weight

1128

Structural formula



Functional category

Non ionic surfactant, emulsifying agent, solubilizing agent, wetting agent.

Application in pharmaceutical formulation

- Used as emulsifying agent in the preparation of oil in water emulsion.
- As a solubilizing agent for various essential oils and oil soluble vitamins.
- It is widely used in cosmetics and food products.

Description

- Have a characteristic odour and warm, slight bitter taste.
- They are yellow oily liquid at 25^{o}C.

Typical properties

Specific gravity	: 1.1
HLB value	: 16.7
Viscosity	: 400 (millipascals)

Solubility

- It is soluble in ethanol and water.
- Insoluble in mineral oil, vegetable oil.

Stability and storage conditions

Stored in a well closed container.

Protected from light, in a cool, dry place.

Incompatabilities

Discoloration and or precipitation occur with various substances like phenols, tannins, tar like substances.

Triton X – 100[70]

Chemical name

Polyoxyethylene octylphenyl ether

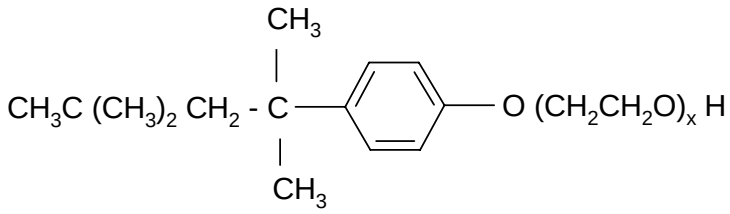
Empirical formula

$C_{34}H_{62}O_{11}$

Molecular weight

646

Structural formula



Functional category

Nonionic surfactant, emulsifying agent, dispersing agent.

Application in pharmaceutical formulation

- They are commonly used in some formulation for emulsion polymerizations.
- It is used as wetting agent for affecting certain staining protocols in microscopy and histology laboratory.

Description

- Viscous liquid

Typical properties

pH: 7 – 9 in a 5% water solution

Solubility

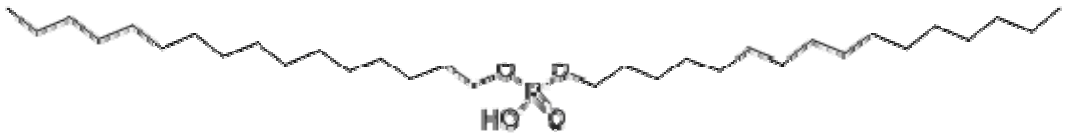
- Miscible with water, alcohol, acetone.
- Soluble in benzene and toluene.
- Insoluble in petroleum ether.

Stability and storage conditions

Stored in a well closed container in a cool, dry place.

DICETYL PHOSPHATE

Structural formula



Functional category:

Stabilising agent

Typical properties:

Melting point - 74-75

Storage temperature - -20

Solubility:

- Soluble or dispersible in oils.
- They are also soluble in most of organic solvents.

Stability and Storage:

Stored in a well closed container in a cool dry place.

5.5. METHODS

5.5.1. Reagent used

Preparation of release media[71]

2.38 gm of disodium hydrogen phosphate, 0.19 gm of potassium dihydrogen phosphate and 8gm of sodium chloride were dissolved in sufficient amount of distilled water to produce 1000ml and pH adjusted to 7.4, if necessary.

5.5.2. Determination of Absorbance maximum (λ_{max})[72]

Zidovidine was dissoled in phosphate buffer saline pH 7.4. Solution with 20 µg/ml concentration was prepared by suitable dilution.

The Zidovudine drug in solution was scanned in UV spectrophotometer from 200 to 400 nm using phosphate buffer saline pH 7.4 as blank. Absorbance maximum was determined as 267 nm. The drug was later quantified by measuring the absorbance at 267 nm in phosphate buffer saline pH7.4.

5.5.3. Standard curve for Zidovudine (by UV metohod)[73]

Preparation of primary stock solution

Zidovudine 100 mg was weighed and dissolved in phosphate buffer saline pH 7.4 in a 100 ml volumetric flask. The flask was shaken and volume was made up to the mark with phosphate buffer saline pH 7.4 to give a solution containing 1000 µg/ml.

Preparation of secondary stock solution

From the primary stock solution, pipette out 2 ml and placed into 100 ml volumetric flask. The volume was made up to mark with phosphate buffer saline pH 7.4 to give a stock solution containing 20 µg / ml.

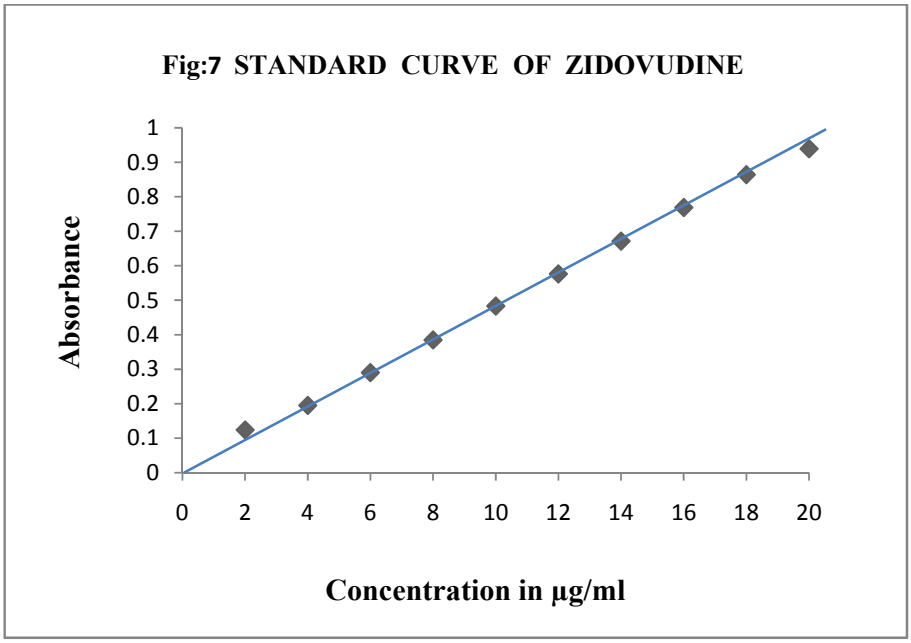
Preparation of sample solution

Appropriate volumes of aliquots (1 to 10 ml) from standard Zidovudine secondary stock solution were transferred to different volumetric flasks of 10 ml capacity. The volume was adjusted to the mark with phosphate buffer saline pH 7.4 to obtain concentrations of 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 µg / ml. Absorbance of each solution against phosphate buffer saline pH 7.4 as blank were measured at 267 nm and the graph of absorbance against concentration were plotted and shown in Figure.8.

Standard curve data for Zidovudine (By U.V method)

Table:4

Concentration in µg/ml	Absorbance at 267 nm
2	0.124
4	0.195
6	0.290
8	0.385
10	0.483
12	0.576
14	0.671
16	0.769
18	0.864
20	0.939



6. RESEARCH ENVISAGED

6.1. INFRARED SPECTROSCOPIC STUDIES [74]

IR study was carried out for identification of pure drug. IR spectroscopy (using Perkin Elmer) by KBr pellet method was carried out on drug. They are compressed under 15 tones pressure in a hydraulic press to form a transparent pellet. The pellet was scanned from 4000 to 400 cm^{-1} in a spectrophotometer and peaks obtained were identified.

6.2. OPTIMIZATION PROCESS FOR NIOSOME PREPARATION

Empty vesicles were prepared by Thin film hydration technique. A liquid phase was prepared by dissolving accurately weighed quantities of surfactant and cholesterol was dissolved in chloroform methanol mixture ratio (2:1v/v) in 100 ml round bottom flask. The solvent mixture was removed from the liquid phase using rotary evaporator at 45-60°C and the speed of rotation was varied like 75rpm, 100rpm, 125rpm and 150rpm to obtain a thin film on the wall of the flask. Simultaneously the residual solvent was completely removed by keeping the flask under the vaccum. The thin film was hydrated with phosphate buffer saline pH 7.4 by varying hydration volume 5ml, 10ml and hydration time of 30, 60 and 120 min at a temperature of 60±2°C. The vesicle size and appearance of empty vesicle were noted.

6.3. FORMULATION OF ZIDOVUDINE NIOSOMES [19]

Zidovudine loaded niosomes were prepared by Thin film hydration technique. Accurately weighed quantity of cholesterol and surfactant were dissolved in chloroform methanol mixture ratio (2:1v/v) in a 100 ml volumetric flask. The weighed quantity of drug and dicetyl phosphate was added to the solvent mixture. The solvent mixture was removed from liquid phase using rotary evaporator at 60°C to obtain a thin film on the wall of the flask at a rotation speed of 150 rpm. The complete removal of solvent can be ensured by applying vaccum. The dry lipid film was hydrated with 5 ml phosphate buffer saline of pH 7.4 at a temperature of 60±2°C for a period of 2 hour until the formation of niosomes. All the batches were subjected to sonication process for 2 min using probe sonicator. The ratios of cholesterol and surfactant used in the formulation were tabulated (table 5).

COMPOSITION OF ZIDOVUDINE NIOSOMES

Table:5

Formulation code	Zidovudine (mg)	Surfactant	Surfactant: Cholesterol (µM)
F_1	10	Span 20	100:100
F_2	10	Span 20	200:100
F_3	10	Span 20	300:100
F_4	10	Span 20	100:200
F_5	10	Span 20	200:200
F_6	10	Span 20	300:200
F_7	10	Span 20	400:200
F_8	10	Tween 20	100:100
F_9	10	Tween 20	200:100
F_{10}	10	Tween 20	300:100
F_{11}	10	Tween 20	100:200
F_{12}	10	Tween 20	200:200
F_{13}	10	Tween 20	300:200
F_{14}	10	Tween 20	400:200

Solvent: chloroform methanol mixture (2:1v/v)

Hydration time: 2 hour

Hydration media: Phosphate buffer saline pH 7.4 (5 ml)

Dicetyl phosphate: 15µM

6.4. EVALUATION OF ZIDOVUDINE NIOSOMES

6.4.1. Removal of unentrapped drug from niosomes [21]

The unentrapped drug from niosomal formulation was separated by centrifugation method. The niosomal suspension was taken in centrifuge tube. The formulation was centrifuged at 15,000 rpm for 30 min using cooling centrifuge and temperature was maintained at 5°c. The supernatant was separated. Supernatant contained unentrapped drug and pellet contained drug encapsulated vesicles. The pellet was resuspended in phosphate buffer saline pH 7.4 to obtain a niosomal suspension free from unentrapped drug.

6.4.2. Encapsulation efficiency

Drug entrapped vesicles were separated from unentrapped drug by centrifugation method. 0.5 ml of zidovudine loaded niosome preparation was added with 0.5 ml of 10% triton X 100 and mixed well then incubated for 1 hour. The triton X 100 was added to lyse the vesicles in order to release the encapsulated zidovudine. The solution was diluted with phosphate buffer saline pH 7.4 and filtered through whatmann filter paper. The filtrate was measured spectrophotometrically at 267 nm using phosphate buffer saline pH 7.4 and triton X 100 mixture as blank.

$$\text{Percent entrapment} = \frac{\text{Amount of drug entrapped}}{\text{Total amount of drug added}} \times 100$$

6.4.3. *In vitro* release study for niosomal preparation [26]

The niosomal formulation was taken in a dialysis membrane of 5 cm length and suitably suspended in a beaker containing 100 ml diffusion medium of phosphate buffer saline pH 7.4. The temperature of medium was maintained at 37±0.5°C. The medium was stirred by means of magnetic stirrer at a constant speed. 1 ml of sample was withdrawn at every 1 hour and replaced with 1 ml of fresh buffer, so that the volume of diffusion medium was maintained constant at 100 ml. The withdrawn samples were made upto 10 ml using phosphate buffer saline pH 7.4. The samples were measured spectrophotometrically at 267 nm.

6.4.4. Zeta potential [22]

The zeta potential of optimized zidovudine niosomal formulation was measured using Malvern zeta potential analyser.

6.4.5. Scanning electron microscopy [27]

The optimized formulation was morphologically characterized by scanning electron microscopy (SEM). The sample for SEM analysis was mounted in the specimen stub using an adhesive small sample was mounted directly in scotsch double adhesive tape. The sample was analysed in hitachi scanning electrom microscope operated at 15 kv and photograph was taken.

6.4.6. Sterility testing [70]

Sterility of prepared and optimized zidovudine niosomal formulation was evaluated by sterility test as per IP. The method selected for testing is Method I – Membrane Filtration Method.

Preparation of Soya bean Casein Digest medium (SCDM)

25 gm of dehydrated media was dissolved in 1000 ml of distilled water. The solution was boiled for 10 min. This solution was used as medium. The medium was cooled to room temperature and pH adjusted to 7.3±0.2. The medium was dispensed in suitable container and sterilized at 121°C for 15 min.

Preparation of Fluid Thioglycollate medium (FTM)

Dehydrated media 26 gm was dissolved in distilled water to get 1000 ml of the medium. It was boiled for 10 min. The medium was cooled to room temperature and pH adjusted to 7.2±0.2. The medium was sterilized at a temperature of 121°C for 15 min. The sterilised media should not have more than upper one- third of the medium in pink colour.

Preparation of Rinsing Fluid (Fluid A)

1 gm of peptic digest of animal tissue was weighed and mixed up with small amount of hot water and made upto 1000 ml. The solution was filtered and pH adjusted to 7.1±0.2. The solution was then dispensed in suitable container and autoclaved for sterilization.

Procedure

The vials containing zidovudine niosomes were broken open under aseptic condition provided by laminar air flow work station. All precaution and preventive measures were taken to avoid contamination by the process or by the analyst. The drug solution was then passed through sterile membrane lodged on a membrane holder assembly. After passing through the solution, the membrane was rinsed three times with 100 ml of sterile peptone (Fluid A). The membrane was cut into two halves using sterile scissors. One half of the filter paper was introduced into the container with SCDM and the other half into the container with FTM.

SCDM containers were then incubated at 22.5±2.5°C and containers at 32.5±2.5°C. The containers were observed for turbidity or appearance of growth of microorganisms for 14 days. Positive control and negative control tests were done to validate the sterility testing procedure.

Negative control

Negative control confirms the sterility of the sterilized media. It was then inoculated and observed for 14 days. Negative control was maintained for both the media and incubated in their appropriate temperature.

Positive control

Positive control confirms the suitability of the media for the growth of microorganism. The positive control for SCDM and FTM were inoculated with Bacillus subtilis suspension and incubated at 22.5±2.5°C and 32.5±2.5°C respectively for 14 days. The growth of microorganisms witnessed by the turbidity of the medium confirms the presence of nourishments favouring the microorganisms.

6.4.7. Stability study of zidovudine niosomes [24]

The optimized zidovudine niosome formulation was examined for stability study. The formulations were taken in a 20 ml sealed glass vial and stored in three different environments such as 4°C, room temperature and 45°c/75% RH for a period of three months. Samples from each batch were withdrawn at the interval of one month and evaluated for entrapment efficiency and *in vitro* drug release.

7. RESULTS AND DISCUSSION

7.1. Development of zidovudine niosomes

In this study, zidovudine loaded niosomes were prepared by Thin film hydration technique using cholesterol and non ionic surfactants such as span 20 and tween 20. Chloroform methanol mixture (2:1v/v) was used as solvent.

After evaporation of solvent from the formulation, thin film was formed. The thin film was hydrated and removed by phosphate buffer saline pH 7.4. Size of the vesicles in formulation was reduced by sonicating the formulation in Probe sonicator.

Formulations with different ratios of surfactant and cholesterol were prepared. Several physicochemical characteristics of niosomes such as morphology, vesicle size determination, drug release profile were investigated. And stability of optimized formulation at various temperatures was evaluated.

Dicetyl phosphate (DCP) also included in the formulation as charge inducing agent. The inclusion of charge inducing agent (DCP) prevented the aggregation and fusion of vesicles. Integrity and uniformity also maintained by dicetyl phosphate.

An effective niosomal drug delivery system should posses good physical and chemical stability on storage and should incorporate high drug loading with stable encapsulation.

7.2. IR studies

Pressed Pellet Technique was used to handle the sample in FTIR spectrometer. In this technique a required amount of sample was added and mixed well with potassium bromide and the mixture was pressed with special discs under high pressure into a transparent pellet and then inserted into special holder of IR spectrometer.

The pellets were scanned from 4000 to 400 cm^{-1} in FTIR spectrophotometer and peaks obtained in both spectrums were identified. The wave number at which peaks appeared and peaks indicating functional groups are presented in table:6.

Table:6

Frequency		**Group assigned**
Pure drug	**Physical mixture**	
3402	3401	OH & NH - stretching
2852	2924	CH – stretching
1660	1654	C=O – stretching
1094	1106	CO – stretching

IR spectrums for pure drug alone and physical mixture of drug, surfactant, and cholesterol were taken. The spectrum of physical mixture was compared with spectrum of pure drug. Bands seen in pure drug also recognized in physical mixture. Hence there was no significant interaction between drug and excepients.

7.3. Optimization of process related variables

The prepared niosomal vesicles were influenced by some factors like speed of rotation, hydration volume, hydration medium and vaccum. Before loading the drug, these factors should be optimized using empty vesicle.

The vaccum used for drying of thin film was 350 mmHg. Vaccum below 350 mmHg was insufficient for complete removal of solvent from the formulation and resulted in aggregation of niosomes on hydration. The vaccum above 350 mmHg resulted rapid evaporation of solvent which leads to entrapment of air bubbles on the surface of film. This caused poor entrapment of drug in niosomes and the vaccum of 350 mmHg produced lipid film had appreciable drug entrapment in the niosomes. Hence 350 mmHg of vaccum was considered as optimum range.

The time of hydration of lipid film was carried from 60 -120 min. When hydration allowed to 120 min, formed niosomes were spherical in shape and existed

in desired size range. So hydration time 120 min and hydration volume 5 ml were selected as optimum.

Thickness and uniformity of thin film was influenced by speed rotation of round bottom flask. The optimum speed was selected to 150 rpm. At this speed of rotation, thin film formed was uniform. The bath temperature of rotary evaporator was maintained at 60±2°C as optimum.

Optimization of process related variables

Table:7

Surfactant: Cholesterol	**Speed of Rotation (rpm)**	**Hydration Time (min)**	**Chloroform: methanol**	**Hydration volume**	**Vesicle Size (µM)**
100:100	75	30	2:1	5 ml	10.29±1.48
	100	60			9.41±1.09
	125	120			9.11±1.88
	150	120			8.69±1.88

Table:8

Volume of hydration medium (ml)	**Hydration time (min)**	**Percentage entrapment (%)**
3	60	60.74 ± 0.98
4	60	68.84 ± 0.76
4	60	73.38 ± 0.58
5	120	89.45 ± 0.88
5	180	81.45 ± 0.93

7.4. Evaluation of Zidovudine Niosomes

7.4.1. Removal of unentrapped drug from Niosomes

The unentrapped drug from niosomes was removed by centrifugation technique. The results are presented in following table,

7.4.2. Percentage drug entrapment efficiency

Table:9

Formulation code	Surfactant: Cholesterol (µM)	Surfactant used	Percentage of free Drug (%)	Percentage entrapment Efficiency (%)
F_1	100:100	Span 20	37	63
F_2	200:100	Span 20	26	74
F_3	300:100	Span 20	32	68
F_4	100:200	Span 20	38	62
F_5	200:200	Span 20	28	72
F_6	300:200	Span 20	16	84
F_7	400:200	Span 20	29	71
F_8	100:100	Tween 20	32	68
F_9	200:100	Tween 20	19	81
F_{10}	300:100	Tween 20	27	73
F_{11}	100:200	Tween 20	29	71
F_{12}	200:200	Tween 20	15	85
F_{13}	300:200	Tween 20	8	92
F_{14}	400:200	Tween 20	24	76

The entrapment efficiency of the niosomes is governed by the ability of formulation to retain drug molecule in aqueous core or in bilayer membrane of vesicles. After removal of unentrapped drug, the entrapment of all formulation was studied. Entrapment efficiency was varied with varying the surfactant and cholesterol ratio. Various factors like lipid concentration, drug to lipid ratio, and cholesterol content will change the entrapment efficiency.

Entrapment efficiency of formulation F_1 was found to be 63%. In formulation F_2, increasing the surfactant concentration, entrapment efficiency was increased to 74%. Further increasing the surfactant concentration in F_3, the entrapment efficiency was decreased to 68%, due to very low concentration of cholesterol.

So, to improve the entrapment efficiency cholesterol concentration was increased to 200µM in formulations F_4, F_5, F_6, and F_7. Because, increase the amount of cholesterol will improve the entrapment efficiency. But entrapment efficiency achieved in formulations F_4, F_5, F_6, and F_7 were 62%, 72%, 84%, and 71%. The drug entrapment was not improved satisfactorily. This is due to, surfactant used in those formulations was span 20. The span 20 is more hydrophobic, hence the hydrophilic drug gets encapsulated in the aqueous core only.

The formulations were tried with tween 20 in formulations F_8, F_9, and F_{10} contained 100 µM of cholesterol and 100, 200, 300 µM of tween 20 respectively. The releases were accordingly, 68%, 81%, and 73%, due to low level of cholesterol concentration.

So quantity of cholesterol was increased 200 µM in formulations F_{11}, F_{12}, F_{13} and F_{14}, entrapment efficiency was improved to 71%, 85%, 92% and 76% respectively. The increase in the entrapment efficiency is attributed to the ability of surfactant and cholesterol to cement the leaking space in the bilayer membrane, which in turn allows enhanced drug level in niosomes. Compared to span 20, the better entrapment efficiency was achieved in tween 20. This can be explained as the tweens are more water soluble, the hydrophilic Zidovudine drug, gets encapsulated as well as partitions into vesicle membrane. Hence formulation F_{13} was optimized one.

7.4.3. *In vitro* release study

The release of Zidovudine from niosomes was determined using the membrane diffusion technique. Release study was carried for 24 hours and results are noted in following tables.

Table:10 *In vitro* drug release for F_1

Time (Hrs)	Amount of drug release (mg)	Cumulative amount of drug release (mg)	Cumulative % drug release (%)
1	0.3	0.3	3.0
2	0.8	0.803	8.03
3	1.5	1.508	15.08
4	1.6	1.615	16.15
5	2.2	2.216	22.16
6	2.4	2.422	24.22
7	2.8	2.824	28.24
8	3.3	3.328	33.28
9	3.5	3.533	35.33
10	4.1	4.135	41.35
11	4.3	4.341	43.41
12	4.7	4.743	47.43
13	4.9	4.947	49.47
14	5.3	5.348	53.48
15	5.8	5.853	58.53
16	6.1	6.158	61.58

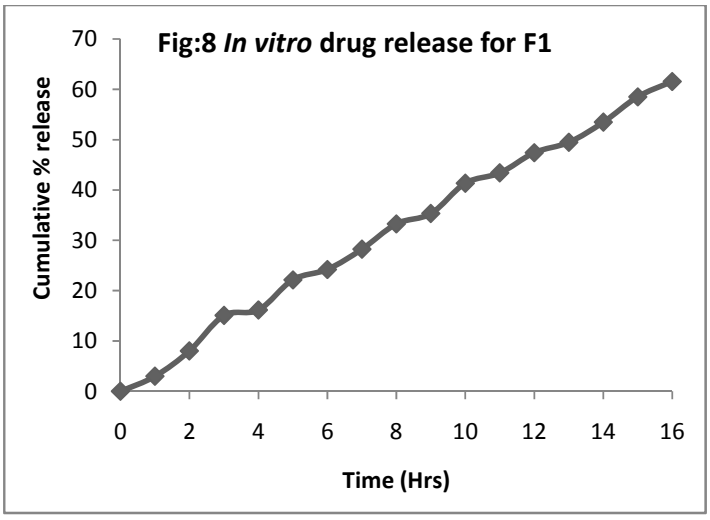


Table:11 *In vitro* drug release for F_2

Time (Hrs)	Amount of drug release (mg)	Cumulative amount of drug release (mg)	Cumulative % drug release (%)
1	0.8	0.8	8.0
2	1.0	1.008	10.08
3	1.1	1.11	11.10
4	1.5	1.511	15.11
5	2.2	2.215	22.15
6	2.6	2.622	26.22
7	3.0	3.026	30.26
8	3.2	3.230	32.30
9	3.3	3.322	33.22
10	3.7	3.733	37.33
11	4.2	4.237	42.37
12	4.8	4.842	48.42
13	5.4	5.448	54.48
14	5.6	5.651	56.51
15	6.8	6.856	68.56
16	6.9	6.968	69.68
17	7.2	7.269	72.69

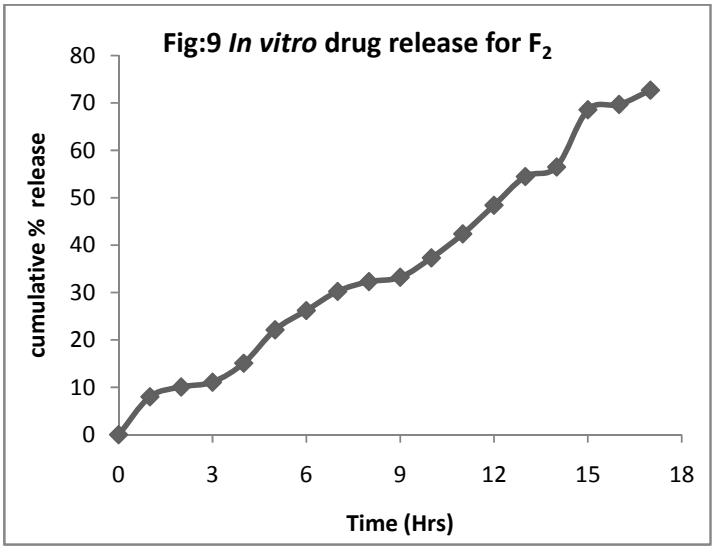


Table:12 *In vitro* drug release for F_3

Time (Hrs)	Amount of drug release (mg)	Cumulative amount of drug release (mg)	Cumulative % drug release (%)
1	0.3	0.3	3.0
2	0.6	0.603	6.03
3	1.4	1.406	14.06
4	1.6	1.614	16.14
5	2.3	2.316	23.16
6	2.7	2.723	27.23
7	2.9	2.927	29.27
8	3.4	3.429	34.29
9	4.8	4.834	48.34
10	4.9	4.948	49.48
11	5.1	5.149	51.49
12	5.4	5.451	54.51
13	5.5	5.554	55.54
14	5.7	5.755	57.55
15	6.4	6.457	64.57
16	6.7	6.764	67.64

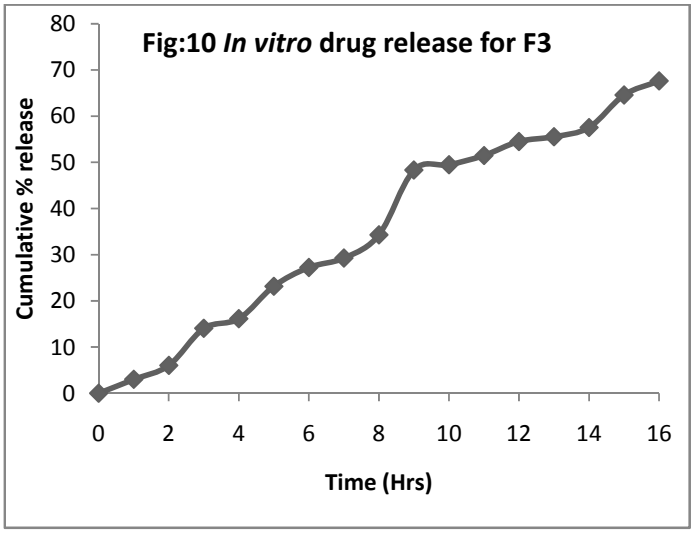


Table:13 *In vitro* drug release for F_4

Time (Hrs)	Amount of drug release (mg)	Cumulative amount of drug release (mg)	Cumulative % drug release (%)
1	0.2	0.2	2.0
2	0.4	0.402	4.02
3	0.9	0.904	9.04
4	1.0	1.009	10.09
5	1.3	1.310	13.10
6	1.4	1.413	14.13
7	1.8	1.814	18.14
8	2.2	2.218	22.18
9	2.5	2.522	25.22
10	2.6	2.625	26.25
11	3.0	3.026	30.26
12	3.1	3.130	31.30
13	3.2	3.231	32.31
14	3.5	3.532	35.32
15	3.6	3.635	36.35
16	3.8	3.836	38.36
17	3.9	3.938	39.38
18	4.0	4.039	40.39
19	4.3	4.340	43.40
20	4.7	4.743	47.43
21	4.9	4.947	49.47
22	5.0	5.049	50.49
23	5.7	5.750	57.50
24	5.9	5.957	59.57

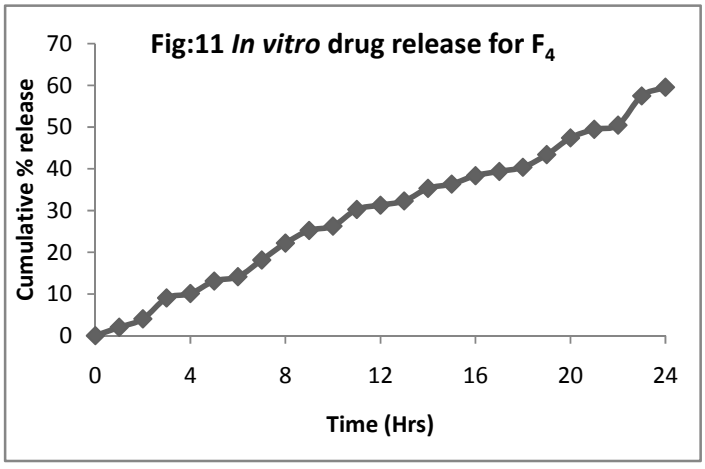


Table:14 *In vitro* drug release for F_5

Time (Hrs)	Amount of drug release (mg)	Cumulative amount of drug release (mg)	Cumulative % drug release (%)
1	0.2	0.2	2.0
2	0.5	0.502	5.02
3	0.7	0.705	7.05
4	0.8	0.807	8.07
5	1.3	1.308	13.08
6	1.5	1.513	15.13
7	1.9	1.915	19.15
8	2.5	2.519	25.19
9	2.6	2.625	26.25
10	3.0	3.026	30.26
11	3.1	3.130	31.30
12	3.3	3.331	33.31
13	3.6	3.633	36.33
14	3.8	3.836	38.36
15	4.2	4.238	42.38
16	4.3	4.342	43.42
17	4.5	4.543	45.43
18	4.8	4.845	48.45
19	5.1	5.148	51.48
20	5.5	5.551	55.51
21	6.0	6.055	60.55
22	6.3	6.360	63.60
23	6.5	6.563	65.63
24	7.1	7.165	71.65

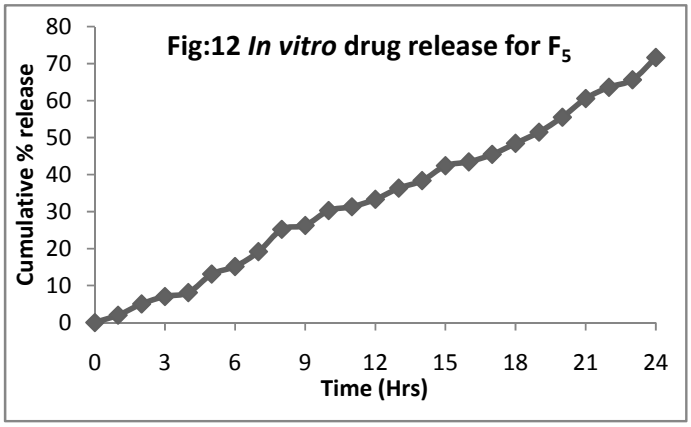


Table:15 *In vitro* drug release for F_6

Time (Hrs)	Amount of drug release (mg)	Cumulative amount of drug release (mg)	Cumulative % drug release (%)
1	0.5	0.5	5.0
2	0.7	0.705	7.05
3	1.0	1.007	10.07
4	1.5	1.510	15.10
5	1.6	1.615	16.15
6	1.8	1.816	18.16
7	2.5	2.518	25.18
8	2.8	2.825	28.25
9	3.3	3.328	33.28
10	3.4	3.433	34.33
11	3.9	3.934	39.34
12	4.1	4.139	41.39
13	4.5	4.541	45.41
14	4.7	4.745	47.45
15	5.5	5.547	55.47
16	5.7	5.755	57.55
17	6.1	6.157	61.57
18	6.6	6.661	66.61
19	6.9	6.966	69.66
20	7.2	7.269	72.69
21	7.4	7.472	74.72
22	7.6	7.674	76.74
23	7.9	7.976	79.76
24	8.1	8.197	81.97

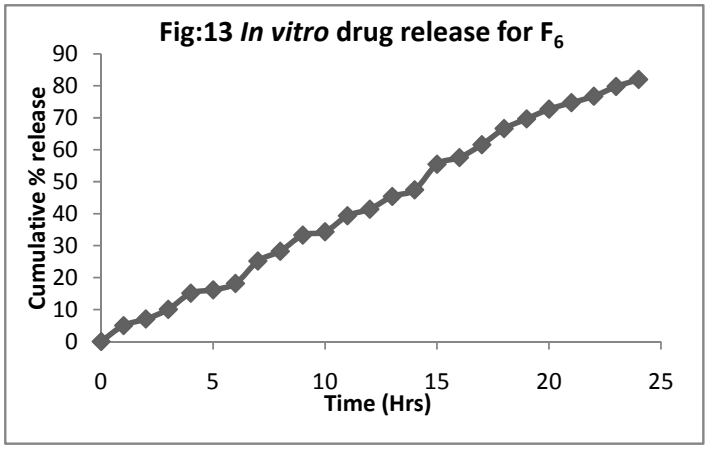


Table:16 *In vitro* drug release for F_7

Time (Hrs)	Amount of drug release (mg)	Cumulative amount of drug release (mg)	Cumulative % drug release (%)
1	0.6	0.6	6.0
2	0.8	0.806	8.06
3	1.5	1.508	15.08
4	1.7	1.715	17.15
5	2.0	2.017	20.17
6	2.1	2.120	21.20
7	2.6	2.621	26.21
8	3.3	3.326	33.26
9	3.4	3.433	34.33
10	4.0	4.034	40.34
11	4.9	4.940	49.40
12	5.5	5.549	55.49
13	6.3	6.355	63.55
14	7.0	7.063	70.63

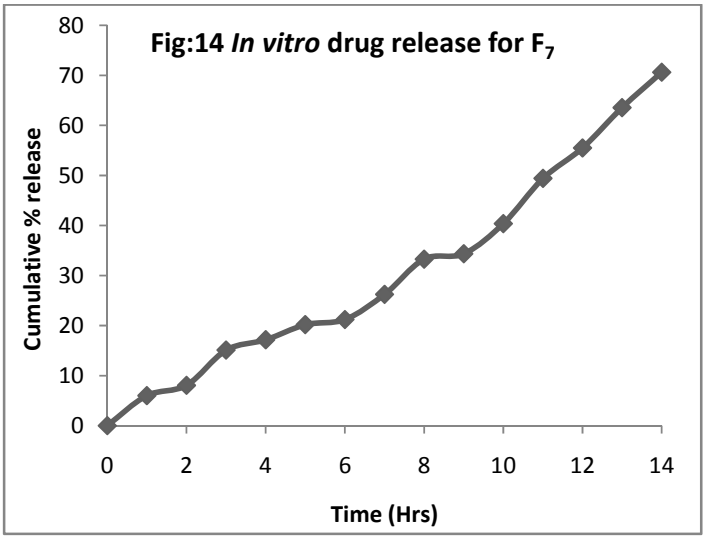


Table:17 *In vitro* drug release for F_8

Time (Hrs)	Amount of drug release (mg)	Cumulative amount of drug release (mg)	Cumulative % drug release (%)
1	0.4	0.4	4.0
2	0.8	0.804	8.04
3	1.1	1.108	11.08
4	1.9	1.911	19.11
5	2.2	2.219	22.19
6	3.4	3.422	34.22
7	3.6	3.634	36.34
8	3.9	3.936	39.36
9	4.1	4.139	41.39
10	4.5	4.541	45.41
11	4.7	4.745	47.45
12	5.0	5.047	50.47
13	5.3	5.35	53.5
14	5.9	5.953	59.53
15	6.1	6.159	61.59
16	6.5	6.561	65.61

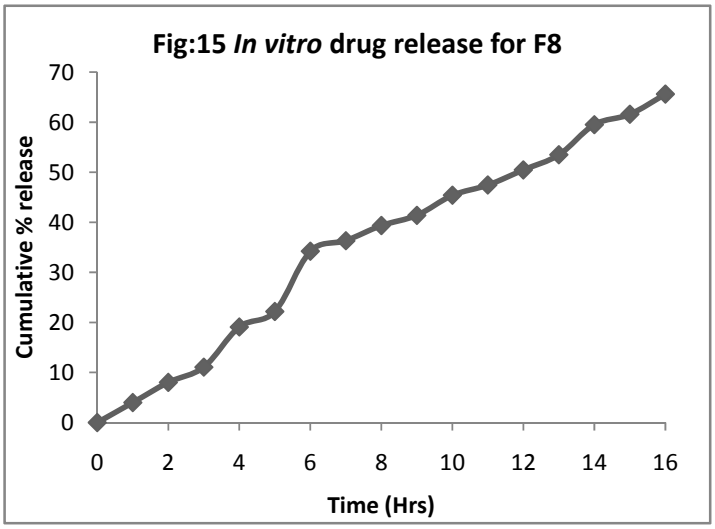


Table:18 *In vitro* drug release for F_9

Time (Hrs)	Amount of drug release (mg)	Cumulative amount of drug release (mg)	Cumulative % drug release (%)
1	0.9	0.9	9.0
2	1.4	1.409	14.09
3	1.5	1.514	15.14
4	1.9	1.915	19.15
5	2.4	2.419	24.19
6	2.9	2.924	29.24
7	3.2	3.229	32.29
8	3.5	3.532	35.32
9	3.6	3.635	36.35
10	4.1	4.136	41.36
11	4.8	4.841	48.41
12	5.1	5.148	51.48
13	5.3	5.351	53.51
14	5.8	5.853	58.53
15	6.1	6.158	61.58
16	6.8	6.861	68.61
17	7.4	7.468	74.68
18	7.9	7.974	79.74

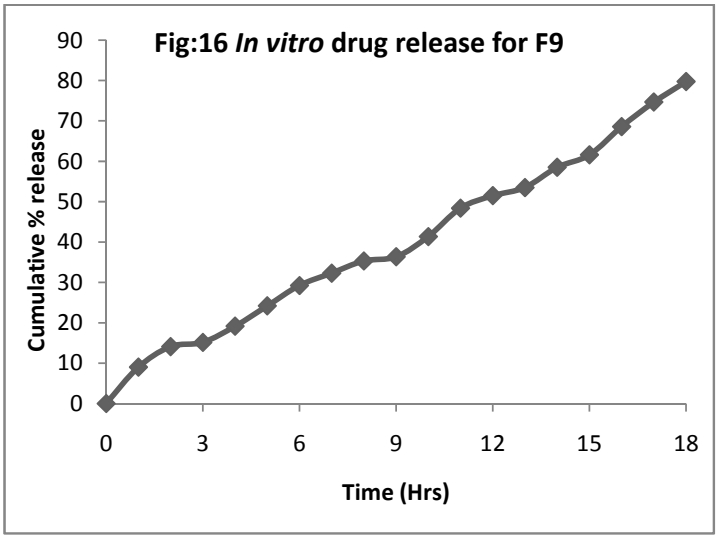


Table:19 *In vitro* drug release for F_{10}

Time (Hrs)	Amount of drug release (mg)	Cumulative amount of drug release (mg)	Cumulative % drug release (%)
1	0.4	0.4	4.0
2	1.5	1.504	15.04
3	1.8	1.815	18.15
4	2.1	2.118	21.18
5	2.8	2.821	28.21
6	3.0	3.028	30.28
7	3.4	3.430	34.30
8	3.9	3.934	39.34
9	4.2	4.239	42.39
10	4.8	4.842	48.42
11	4.9	4.948	49.48
12	5.3	5.349	53.49
13	5.8	5.853	58.53
14	5.9	5.958	59.58
15	6.3	6.359	63.59
16	6.6	6.663	66.63
17	6.8	6.866	68.66
18	7.1	7.168	71.68

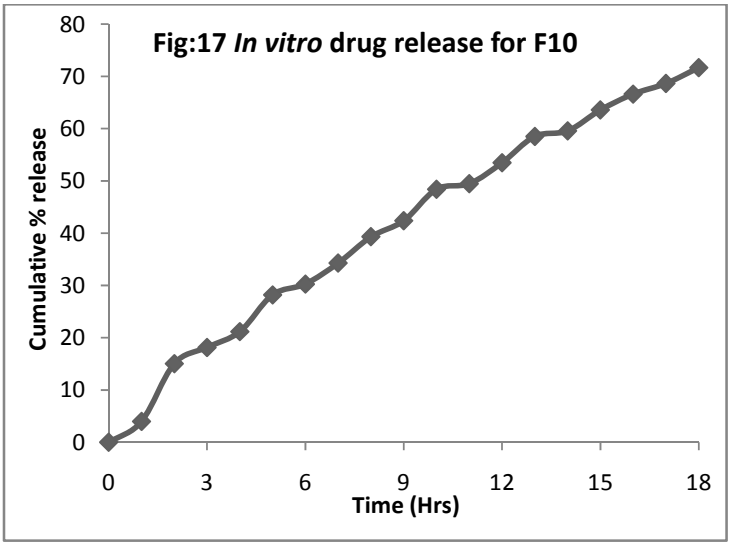


Table:20 *In vitro* drug release for F_{11}

Time (Hrs)	Amount of drug release (mg)	Cumulative amount of drug release (mg)	Cumulative % drug release (%)
1	0.40	0.40	4.0
2	0.5	0.504	05.04
3	0.8	0.805	08.05
4	1.1	1.108	11.08
5	1.2	1.211	12.11
6	1.5	1.512	15.12
7	1.9	1.915	19.15
8	2.4	2.419	24.19
9	2.5	2.524	25.24
10	3.0	3.025	30.25
11	3.1	3.130	31.30
12	3.3	3.331	33.31
13	3.9	3.933	39.33
14	4.0	4.039	40.39
15	4.2	4.240	42.40
16	4.6	4.642	46.42
17	4.7	4.746	47.46
18	5.0	5.047	50.47
19	5.1	5.150	51.50
20	5.3	5.351	53.51
21	5.7	5.753	57.53
22	5.8	5.857	58.57
23	6.1	6.158	61.58
24	6.9	6.961	69.61

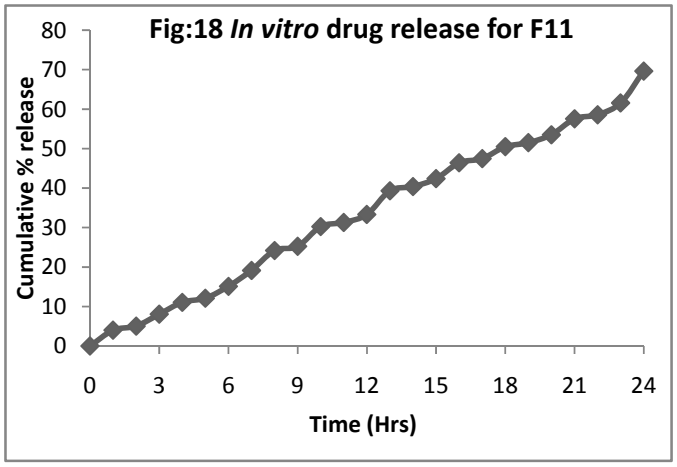


Table:21 *In vitro* drug release for F_{12}

Time (Hrs)	Amount of drug release (mg)	Cumulative amount of drug release (mg)	Cumulative % drug release (%)
1	0.8	0.8	8.0
2	1.0	1.008	10.08
3	1.5	1.510	15.10
4	1.6	1.615	16.15
5	1.8	1.816	18.16
6	2.3	2.318	23.18
7	2.6	2.623	26.23
8	2.8	2.826	28.26
9	3.3	3.328	33.28
10	3.5	3.533	35.33
11	3.9	3.935	39.35
12	4.0	4.039	40.39
13	4.4	4.440	44.40
14	4.6	4.644	46.44
15	5.1	5.146	51.46
16	5.5	5.551	55.51
17	6.0	6.055	60.55
18	6.6	6.660	66.60
19	6.7	6.766	67.66
20	6.9	6.967	69.67
21	7.1	7.169	71.69
22	7.2	7.271	72.71
23	7.8	7.872	78.72
24	8.3	8.378	83.78

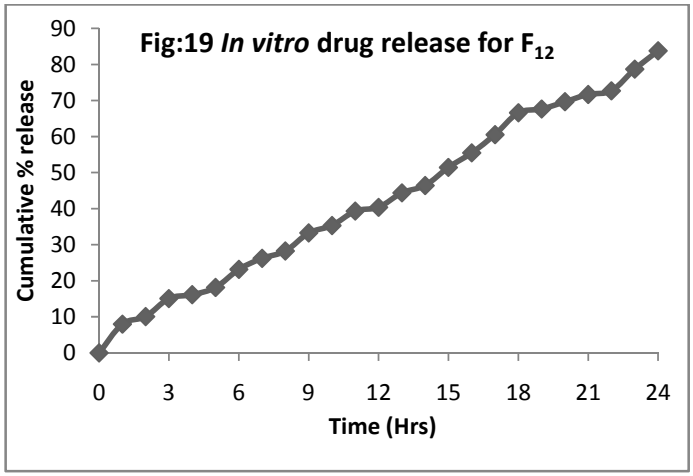


Table:22 *In vitro* drug release for F_{13}

Time (Hrs)	Amount of drug release (mg)	Cumulative amount of drug release (mg)	Cumulative % drug release (%)
1	1.2	1.20	12.0
2	1.9	1.912	19.12
3	2.1	2.119	21.19
4	2.3	2.321	23.21
5	2.4	2.423	24.23
6	2.9	2.924	29.24
7	3.1	3.129	31.29
8	3.9	3.931	39.31
9	4.1	4.139	41.39
10	4.4	4.441	44.41
11	4.8	4.844	48.44
12	5.0	5.048	50.48
13	5.2	5.250	52.50
14	5.4	5.452	54.52
15	5.7	5.754	57.54
16	5.8	5.857	58.57
17	6.4	6.458	64.58
18	6.8	6.864	68.64
19	6.9	6.968	69.68
20	7.5	7.569	75.69
21	7.9	7.975	79.75
22	8.4	8.479	84.79
23	8.6	8.684	86.84
24	9.0	9.086	90.86

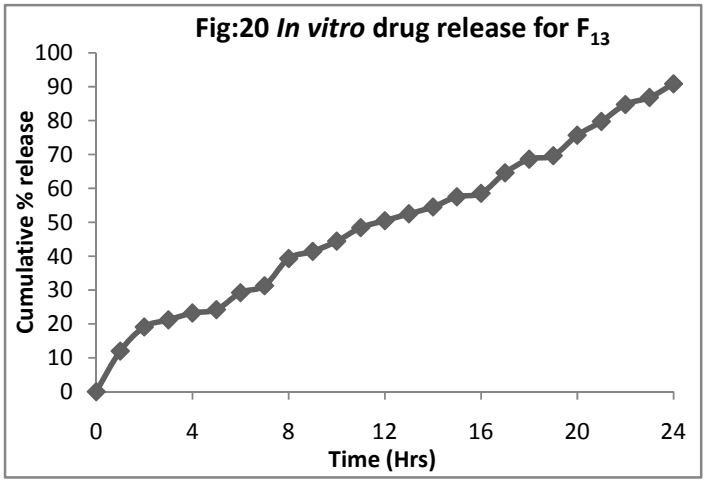
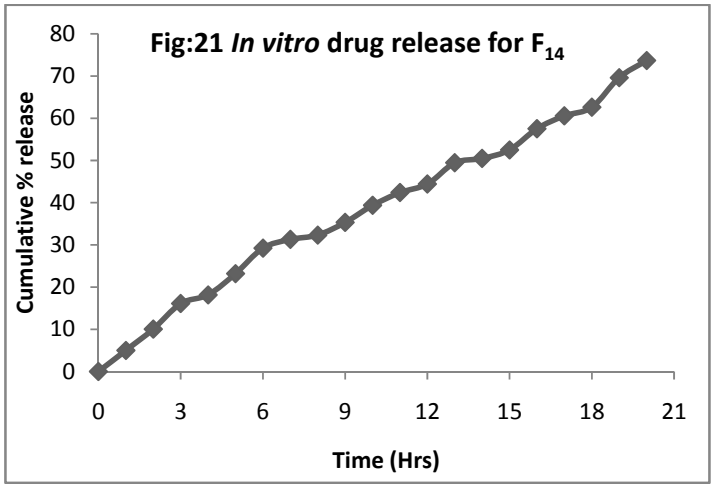


Table:23 *In vitro* drug release for F_{14}

Time (Hrs)	Amount of drug release (mg)	Cumulative amount of drug release (mg)	Cumulative % drug release (%)
1	0.5	0.5	5.0
2	1.0	1.005	10.05
3	1.6	1.610	16.10
4	1.8	1.816	18.16
5	2.3	2.318	23.18
6	2.9	2.923	29.23
7	3.1	3.129	31.29
8	3.2	3.231	32.31
9	3.5	3.532	35.32
10	3.9	3.935	39.35
11	4.2	4.239	42.39
12	4.4	4.442	44.42
13	4.9	4.944	49.44
14	5.0	5.049	50.49
15	5.2	5.250	52.50
16	5.7	5.752	57.52
17	6.0	6.057	60.57
18	6.2	6.260	62.60
19	6.9	6.962	69.62
20	7.3	7.369	73.69



***In vitro* release study of zidovudine niosomes**

Table:24

Formulation code	**Surfactant: Cholesterol (µM)**	**Surfactant used**	**Total release period (Hrs)**	**Cumulative percentage drug release (%)**
F_1	100:100	Span 20	16	61.58
F_2	200:100	Span 20	17	72.69
F_3	300:100	Span 20	16	67.64
F_4	100:200	Span 20	24	59.57
F_5	200:200	Span 20	24	71.65
F_6	300:200	Span 20	24	81.97
F_7	400:200	Span 20	14	70.63
F_8	100:100	Tween 20	16	65.61
F_9	200:100	Tween 20	18	79.74
F_{10}	300:100	Tween 20	18	71.68
F_{11}	100:200	Tween 20	24	69.61
F_{12}	200:200	Tween 20	24	83.78
F_{13}	300:200	Tween 20	24	90.86
F_{14}	400:200	Tween 20	20	73.69

In vitro drug release was carried out for 24 hours using phosphate buffer as diffusion medium. It was found to be biphasic, and the release was controlled by lipid bilayer and dialysis membrane. Incorporation of cholesterol affected the release rate of encapsulated drug. *In vitro* drug release characteristics for formulations containing two different surfactants were compared. Zidovudine niosomes were tried with two different surfactant and cholesterol concentrations.

Drug release from formulations F_1, F_2 and F_3 were found to be 61.58%, 72.69%, and 67.64% in 16 hrs, 17 hrs and 16 hrs respectively. The release was not extended upto 24 hrs, because those formulations contained low cholesterol concentration.

Quantity of cholesterol was increased to 200µM in formulations F_4, F_5, F_6 and F_7 and release was achieved to 59.57% in 24 hrs, 71.65% in 24 hrs, 81.97% in 24 hours and 70.63% in 14 hrs. Except F_7, release from other formulation was extended to 24 hrs. This is due to higher concentration of surfactant in formulation F_7. Extended release was achieved but those formulations were not satisfied with percentage drug release. Higher release was found to be 81.83%. This is due to the water insoluble nature of span 20.

Due to lower concentration of cholesterol in formulations F_8, F_9 and F_{10}, the release were 65.61% 16 hrs, 79.48% at 18 hrs and 71.68% at 18 hrs respectively. Formulations F_{11}, F_{12}, F_{13} and F_{14} contained 200 µM of cholesterol showed 69.61% of drug release in 24 hrs, 83.78% of drug release in 24 hrs, 90.86% of drug release in 24 hrs and 73.69% of drug release in 20 hrs. Higher release of 90.86% was found in formulation contained 300:200 µmol ratio of surfactant and cholesterol. So formulation F_{13} (300:200 µmol) was considered as optimized formulation.

Table:25 *In vitro* drug release studies for formulations containing Span 20

Time (Hrs)	Cumulative percentage drug release (%)						
	F_1	F_2	F_3	F_4	F_5	F_6	F_7
1	3.0	8.0	3.0	2.0	2.0	5.0	6.0
2	8.03	10.08	6.03	4.02	5.02	7.05	8.06
3	15.08	11.10	14.06	9.04	7.05	10.07	15.08
4	16.15	15.11	16.14	10.09	8.07	15.10	17.15
5	22.16	22.15	23.16	13.10	13.08	16.15	20.17
6	24.22	26.22	27.23	14.13	15.13	18.16	21.20
7	28.24	30.26	29.27	18.14	19.15	25.18	26.21
8	33.28	32.30	34.29	22.18	25.19	28.25	33.26
9	35.33	33.22	48.34	25.22	26.25	33.28	34.33
10	41.35	37.33	49.48	26.25	30.26	34.33	40.34
11	43.41	42.37	51.49	30.26	31.30	39.34	49.40
12	47.43	48.42	54.51	31.30	33.31	41.39	55.49
13	49.47	54.48	55.54	32.31	36.33	45.41	63.55
14	53.48	56.51	57.55	35.32	38.36	47.45	70.63
15	58.53	68.56	64.57	36.35	42.38	55.47	-
16	61.58	69.68	67.64	38.36	43.42	57.55	-
17	-	72.69	-	39.38	45.43	61.57	-
18	-	-	-	40.39	48.45	66.61	-
19	-	-	-	43.40	51.48	69.66	-
20	-	-	-	47.43	55.51	72.69	-
21	-	-	-	49.47	60.55	74.72	-
22	-	-	-	50.49	63.60	76.74	-
23	-	-	-	57.50	65.63	79.76	-
24	-	-	-	59.57	71.65	81.97	-

Fig:22 Comparative *in vitro* release study of zidovudine niosomal formulations

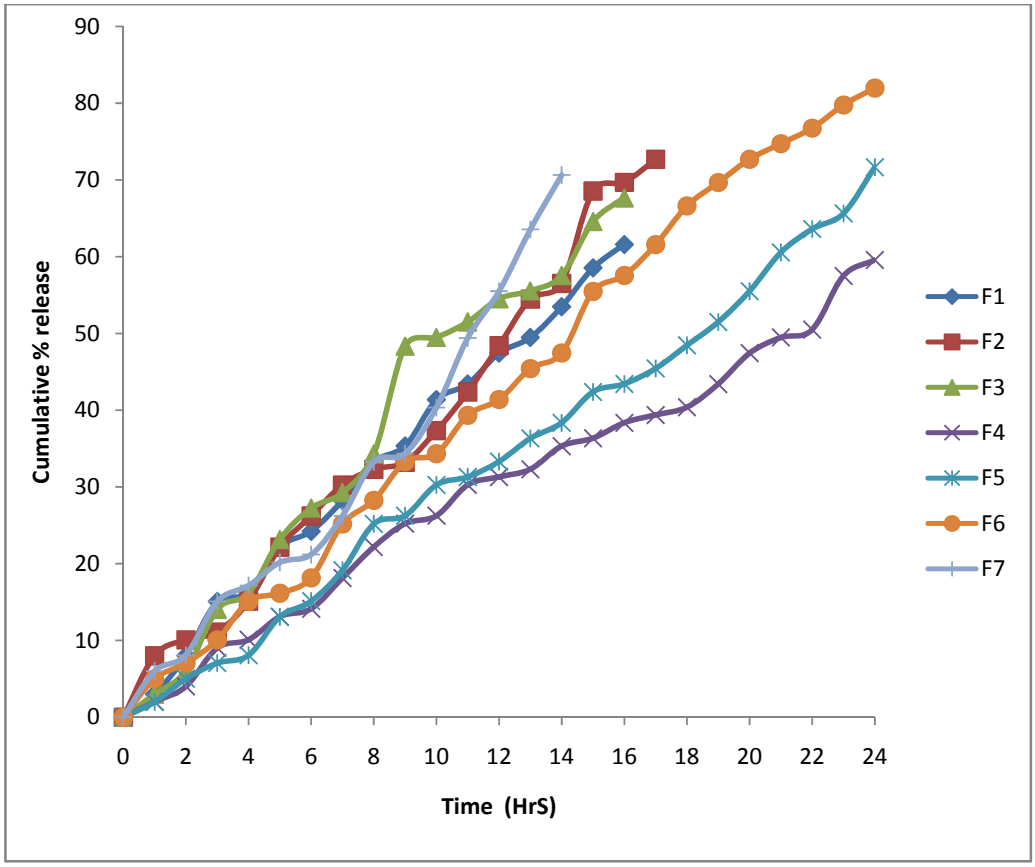
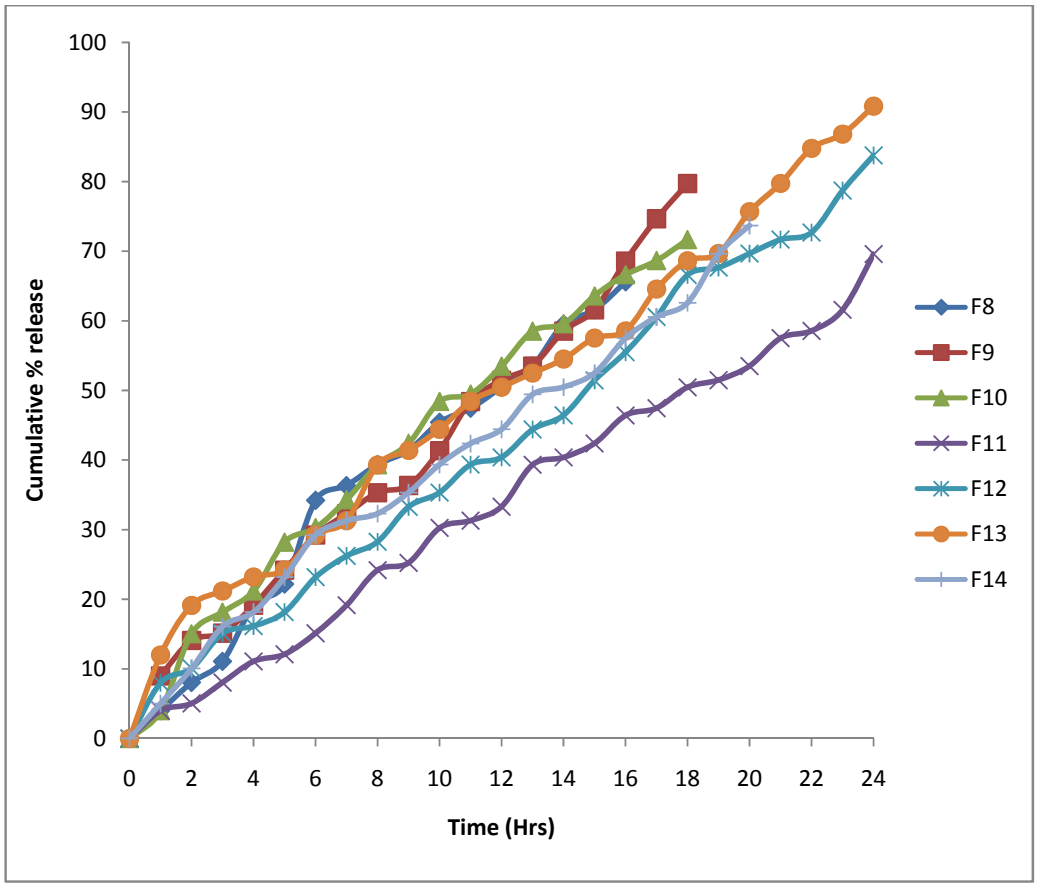


Table:26 *In vitro* drug release studies for formulations containing Tween 20

Time (Hrs)	Cumulative percentage drug release (%)						
	F_1	F_2	F_3	F_4	F_5	F_6	F_7
1	4.0	9.0	4.0	4.0	8.0	12.0	5.0
2	8.04	14.09	15.04	05.04	10.08	19.12	10.05
3	11.08	15.14	18.15	08.05	15.10	21.19	16.10
4	19.11	19.15	21.18	11.08	16.15	23.21	18.16
5	22.19	24.19	28.21	12.11	18.16	24.23	23.18
6	34.22	29.24	30.28	15.12	23.18	29.24	29.23
7	36.34	32.29	34.30	19.15	26.23	31.29	31.29
8	39.36	35.32	39.34	24.19	28.26	39.31	32.31
9	41.39	36.35	42.39	25.24	33.28	41.39	35.32
10	45.41	41.36	48.42	30.25	35.33	44.41	39.35
11	47.45	48.41	49.48	31.30	39.35	48.44	42.39
12	50.47	51.48	53.49	33.31	40.39	50.48	44.42
13	53.5	53.51	58.53	39.33	44.40	52.50	49.44
14	59.53	58.53	59.58	40.39	46.44	54.52	50.49
15	61.59	61.58	63.59	42.40	51.46	57.54	52.50
16	65.61	68.61	66.63	46.42	55.51	58.57	57.52
17	-	74.68	68.66	47.46	60.55	64.58	60.57
18	-	79.74	71.68	50.47	66.60	68.64	62.60
19	-	-	-	51.50	67.66	69.68	69.62
20	-	-	-	53.51	69.67	75.69	73.69
21	-	-	-	57.53	71.69	79.75	-
22	-	-	-	58.57	72.71	84.79	-
23	-	-	-	61.58	78.72	86.84	-
24	-	-	-	69.61	83.78	90.86	-

Fig:23 Comparative *in vitro* release study of zidovudine niosomal formulations



7.4.4. Scanning electron microscopy

The surface characteristics of zidovudine niosomal formulation were studied by scanning electron microscopy. SEM image of prepared niosome formulation shows that the coating of surfactant cholesterol mixture on drug particles. Some particles in the images are broken, which might be due to handling and processing. Most of the vesicles are spherical and discrete sharp boundaries. The appearance of niosome vesicles in scanning electron micrograph is smooth, which indicates a thin and uniform coating over the drug. Based on the scale of micrograph, no significant change in size of particles is seen. The observation clearly shows that, there is no aggregation between the particles, due to surfactant coating.

7.4.5. Zeta potential

The addition of membrane additives affects zeta potential value depending on the type of membrane additives. Zeta potential of optimized zidovudine niosome formulation was measured and found to -27.3 mv. The negative zeta potential observed with niosomes reflects the presence of negatively charged DCP on the surface of vesicles. The obtained result of the zeta potential of the prepared formulation indicates particles in the formulation remains suspended and so were found to be stable. The particles being suspended. The formulation was found to be very effective for parenteral administration.

7.4.6. Sterility test

The optimized Zidovudine niosomal formulation was subjected to sterility test. The test was carried out as per I.P specification. Both Soya bean casein digest medium (SCDM) and Fluid thioglycollate medium (FTM) were used. The method followed is Method A – Membrane filtration method. The positive control was prepared from standardised Bacillus subtilis suspension. The samples dipped in SCDM and FTM incubated for 14 days. The absence of turbidity of the test indicates the sterility of the formulation and passed the sterility test.

7.4.7. Stability studies

The optimized zidovudine niosomal formulation (F_{13}) was subjected to stability study for three months at 4^0C, room temperature and 45^0C/75%RH. At the interval of one month the niosomes evaluated for *in vitro* release and entrapment efficiency. The stability study shows that niosomal formulations are more stable at 4°C (refrigerator) when compared to room temperature and at 45^0C/75%RH

Table:27 Entrapment efficiency data

Temperature	Percentage entrapment after one month (%)	Percentage entrapment after two months (%)	Percentage entrapment after three months (%)
4°C	90	89	89
Room temperature	87	85	80
45°C/75% RH	84	81	74

Table:28 *In vitro* release data

Temperature	Cumulative % release after one month (%)	Cumulative % release after two months (%)	Cumulative % release after three months (%)
4°C	89.87	88.84	87.83
Room temperature	85.83	82.80	78.77
45°C/75% RH	81.79	75.73	70.66

Table:29 *In vitro* data for optimized formulation F_{13} stability study at 4^0C

Time (Hrs)	Cumulative % drug release		
	1st month (%)	2nd month (%)	3rd month (%)
1	7.0	4.0	8.0
2	9.07	8.04	10.08
3	13.09	13.08	11.10
4	19.13	15.13	17.11
5	24.19	16.15	19.17
6	25.24	22.16	23.19
7	32.24	25.22	24.23
8	39.32	29.25	28.24
9	48.39	35.29	31.28
10	50.48	42.35	32.31
11	54.50	44.42	35.32
12	56.54	49.44	39.35
13	60.56	50.49	44.39
14	63.60	53.50	45.44
15	65.63	59.53	52.44
16	69.65	61.59	59.52
17	72.69	63.61	63.59
18	75.72	70.63	64.63
19	77.75	72.70	68.64
20	81.77	74.72	74.68
21	82.81	78.74	75.74
22	83.82	80.78	79.75
23	87.83	84.80	83.79
24	89.87	88.84	87.83

Fig:27 STABILITY STUDY RELEASE DATA FOR FORMULATION F_{13} AFTER THREE MONTHS AT 4°C

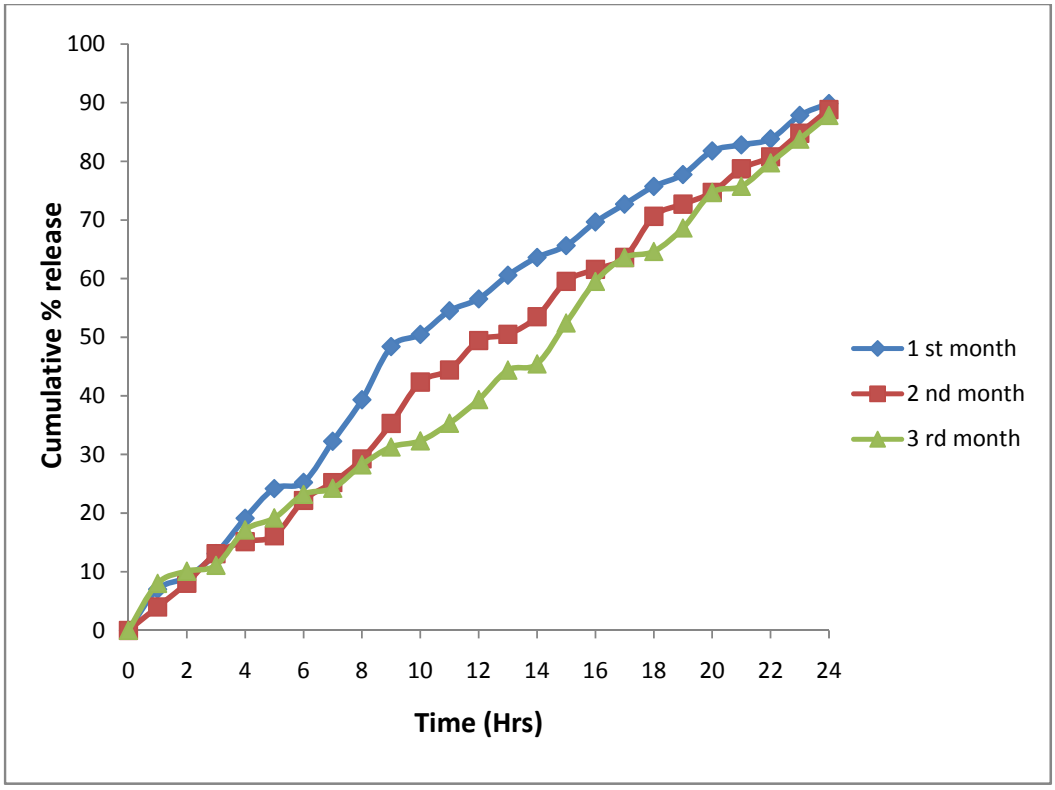


Table:30 *In vitro* data for optimized formulation F_{13} stability study at Room temperature

Time (Hrs)	Cumulative % drug release		
	1st month (%)	2nd month (%)	3rd month (%)
1	7.0	5.0	5.0
2	9.07	7.05	7.05
3	12.09	13.07	14.07
4	16.12	17.13	20.14
5	19.08	24.17	22.20
6	23.19	29.24	26.22
7	24.23	31.29	27.26
8	29.24	34.31	32.27
9	33.29	39.34	34.32
10	34.33	45.39	37.34
11	39.34	47.45	39.37
12	41.39	49.47	42.38
13	45.41	51.49	44.42
14	47.45	53.51	48.42
15	49.47	56.53	50.48
16	50.49	61.56	51.50
17	62.50	63.61	58.51
18	63.62	68.63	60.58
19	69.63	69.68	66.60
20	70.69	71.69	67.66
21	76.70	72.71	69.67
22	79.76	77.72	74.69
23	83.79	80.77	77.74
24	85.83	82.80	78.77

Fig:28 STABILITY STUDY RELEASE DATA FOR FORMULATION F_{13} AFTER THREE MONTHS AT ROOM TEMPERATURE

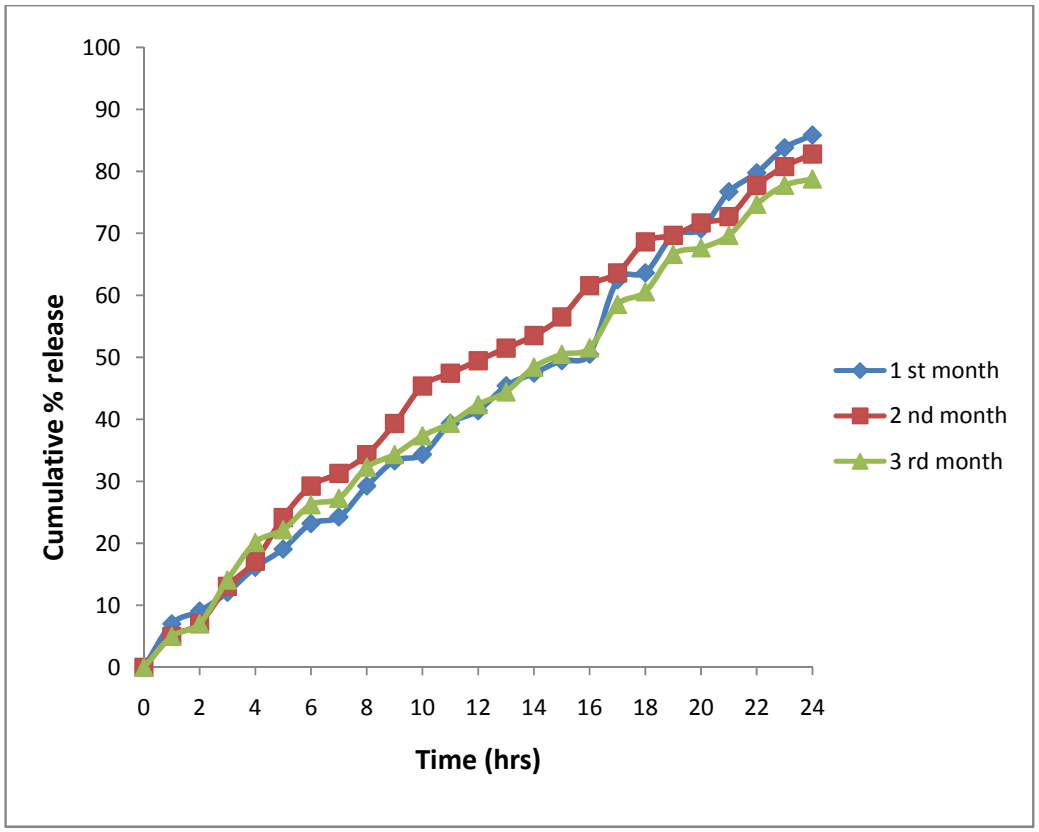
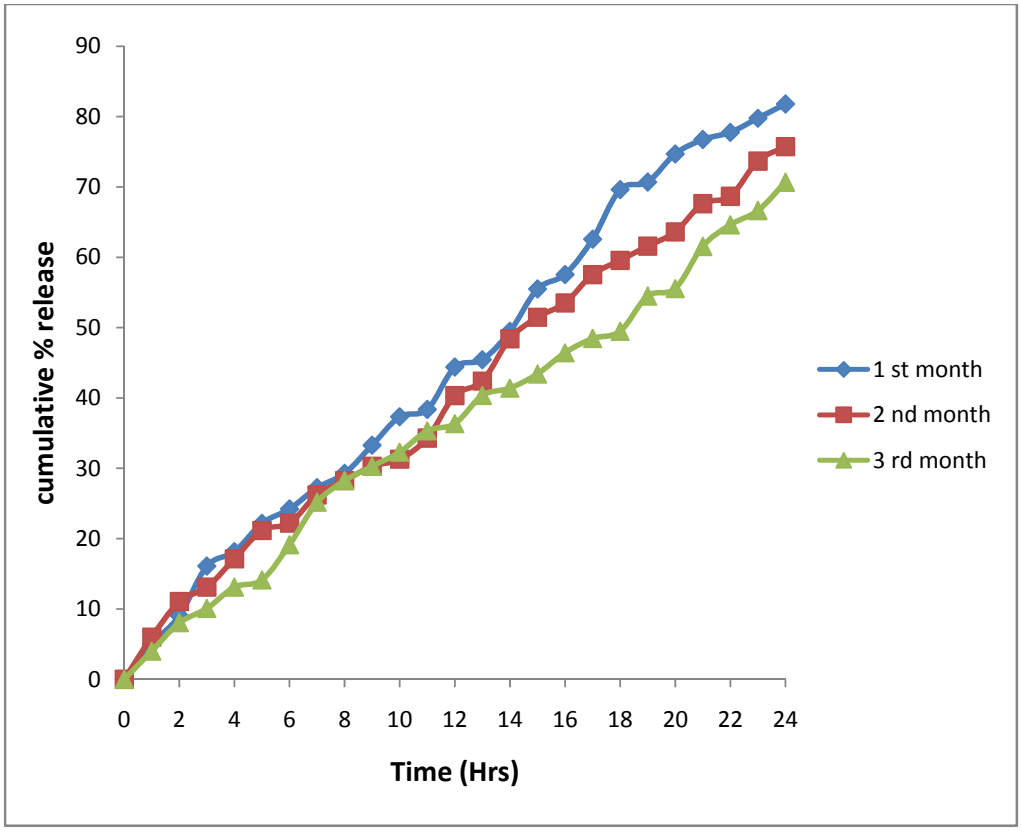


Table:31 *In vitro* data for optimized formulation F_{13} stability study at 45°c/75%RH

Time (Hrs)	Cumulative % drug release		
	1st month (%)	2nd month (%)	3rd month (%)
1	5.0	6.0	4.0
2	9.05	11.06	8.04
3	16.09	13.11	10.08
4	18.16	17.13	13.10
5	22.18	21.17	14.13
6	24.22	22.21	19.14
7	27.24	26.22	25.19
8	29.27	28.26	28.25
9	33.29	30.28	30.28
10	37.33	31.30	32.30
11	38.37	34.31	35.32
12	44.38	40.34	36.35
13	45.44	42.40	40.36
14	49.45	48.42	41.40
15	55.49	51.48	43.41
16	57.55	53.51	46.43
17	62.57	57.53	48.45
18	69.62	59.57	49.48
19	70.69	61.59	54.49
20	74.70	63.61	55.54
21	76.74	67.63	61.55
22	77.76	68.67	64.61
23	79.77	73.68	66.64
24	81.79	75.73	70.66

Fig:29 STABILITY STUDY RELEASE DATA FOR FORMULATION F_{13} AFTER THREE MONTHS AT 45°C /75% RH



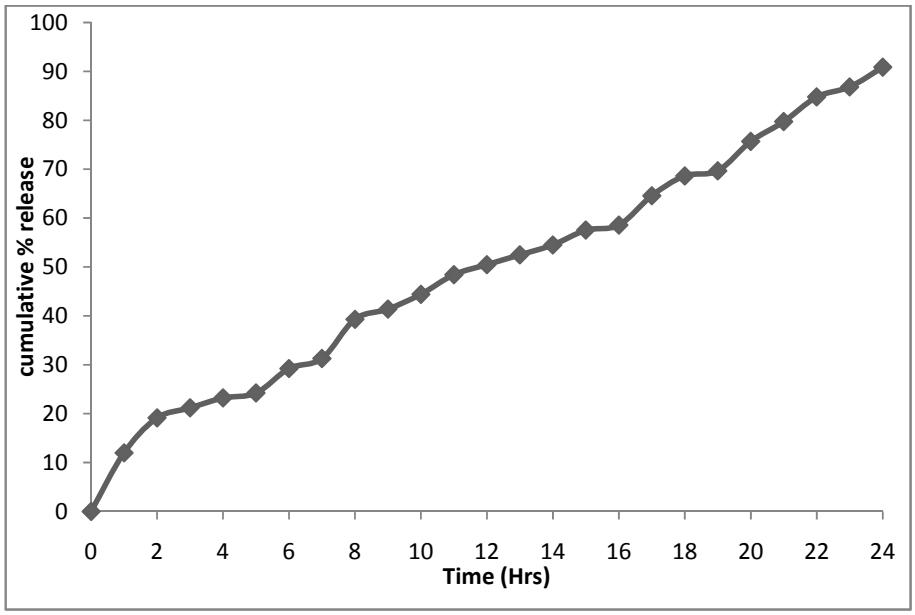
7.4.8. Kinetics of drug release

The optimized formulation F_{13} was subjected to graphical treatment to assess the kinetics of drug release.

ZERO ORDER PLOT

The optimized formulation F_{13} is most suitable parenteral administration as it founds to be good in the *in vitro* release kinetic study. The zero order plot obtained by plotting cumulative percentage drug release versus time. The regression value is 0.9887.

Fig:30 Zero order plot for formulation F_{13}



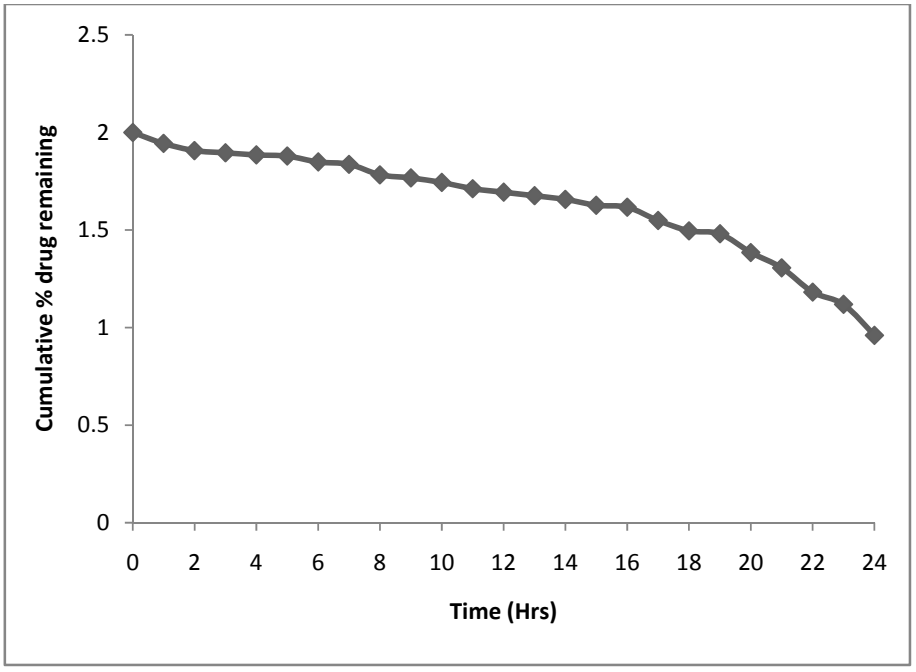
Slope = 3.3753

Regression = 0.9887

FIRST ORDER PLOT

The first order plot was obtained by plotting log cumulative percentage of drug remaining versus time. The regression value is 0.889.

Fig:31 First order plot for formulation F_{13}



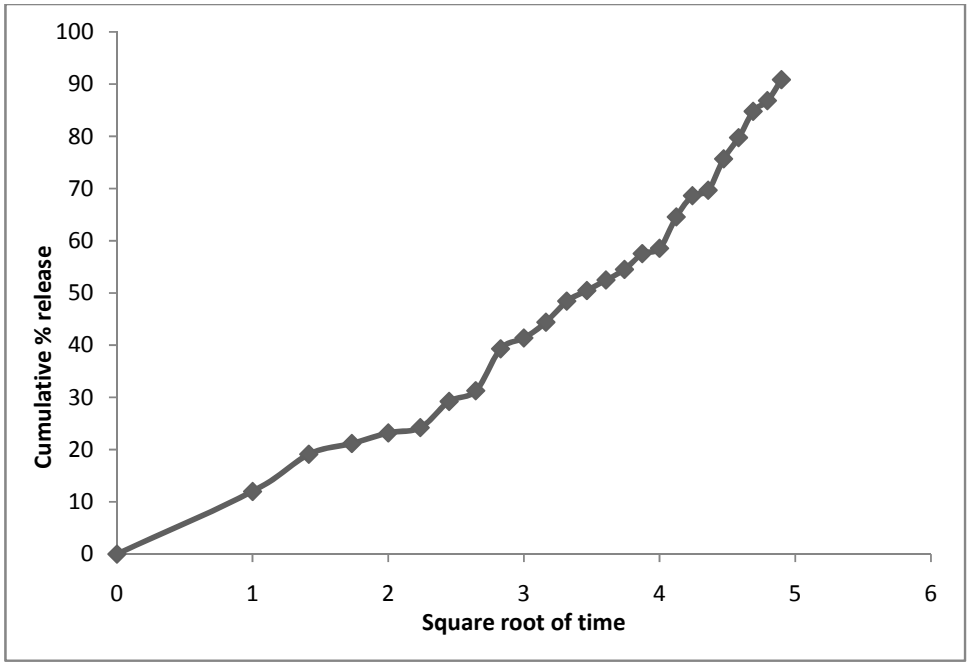
Slope = -0.035

Regression = 0.889

HIGUCHI PLOT

The Higuchi plot was made by plotting cumulative percentage drug release versus square root of time. The regression value is 0.9539. It confirms that the release is diffusion mediated.

Fig:32 Higuchi plot for formulation F_{13}



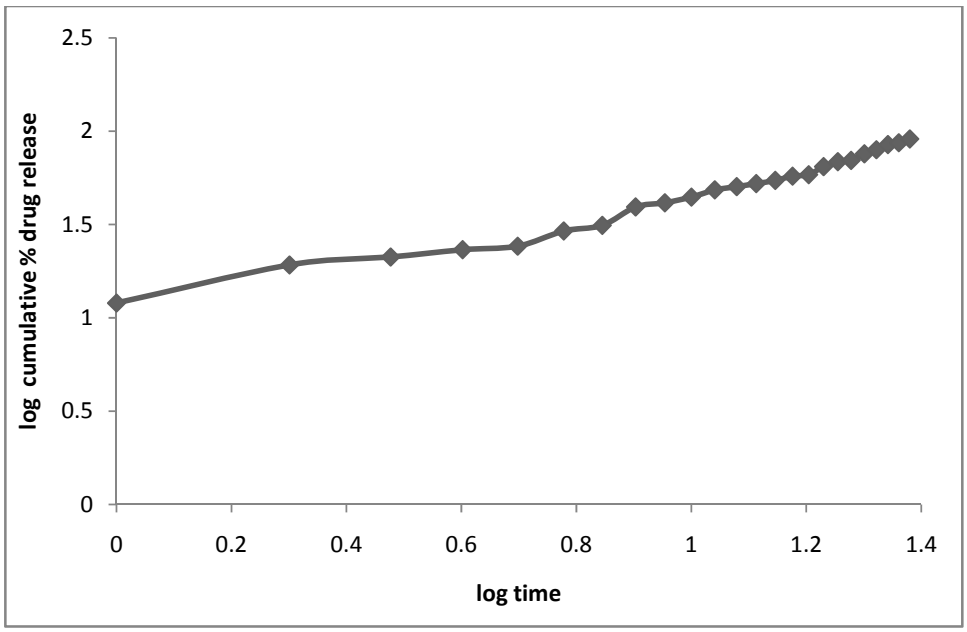
Slope = 1.8921

Regression = 0.9539

KORSEMEYER PLOT

The graph was obtained by log cumulative percentage drug release versus log time. The n value is 0.6579. The n value ($0.45 < n < 0.89$) indicates that the drug release follows anomalous (non fickian) diffusion.

Fig:33 Korsemeyer plot for formulation F_{13}



n = 0.6579

Regression = 0.9761

Fig:24 Scanning Electron Microscopy

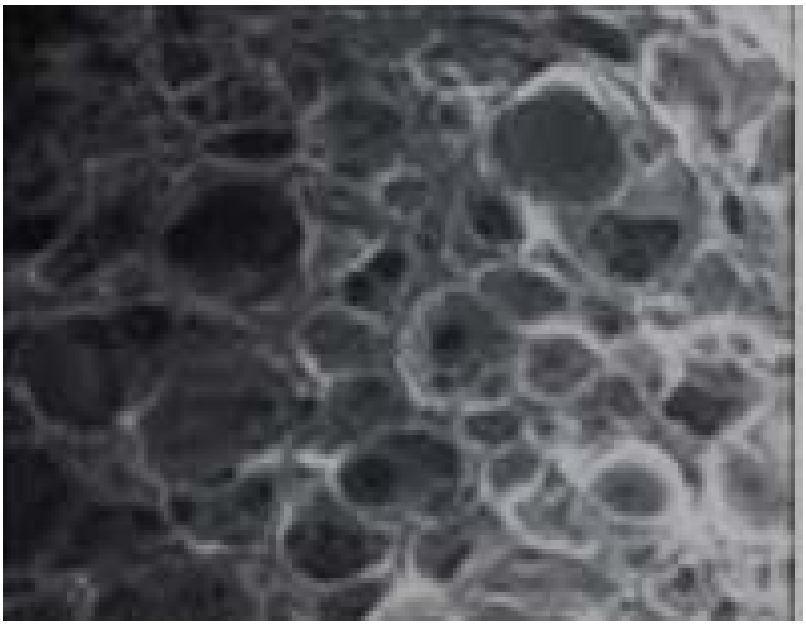
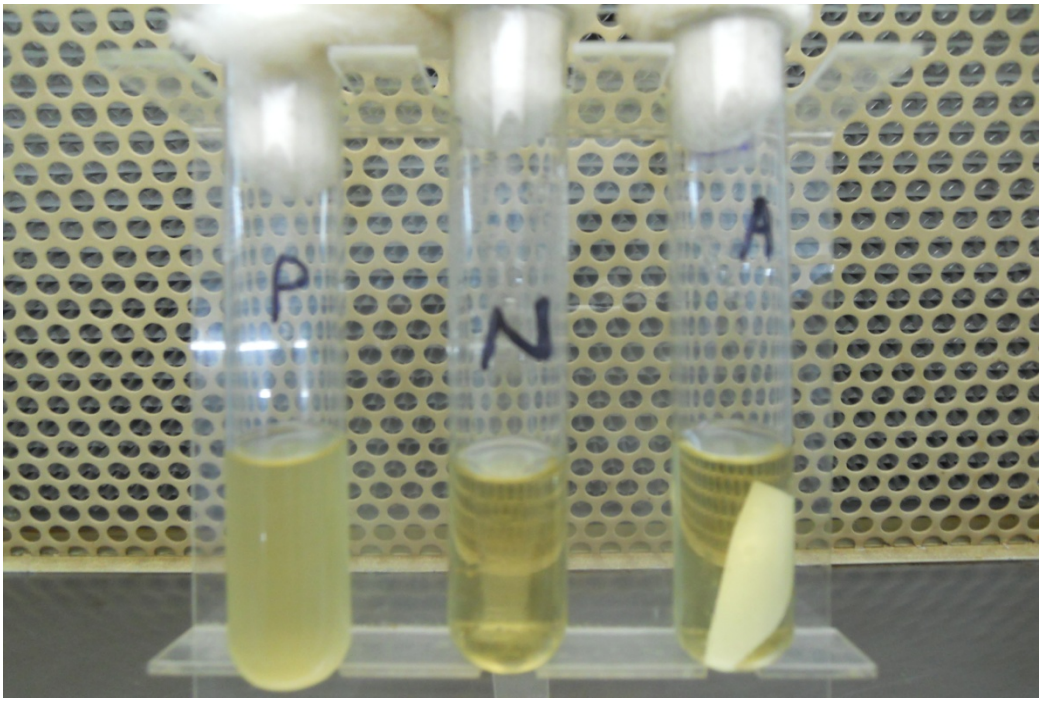


Fig:25 Observation of the sterility test done in soyabean casein digest medium (SCDM).

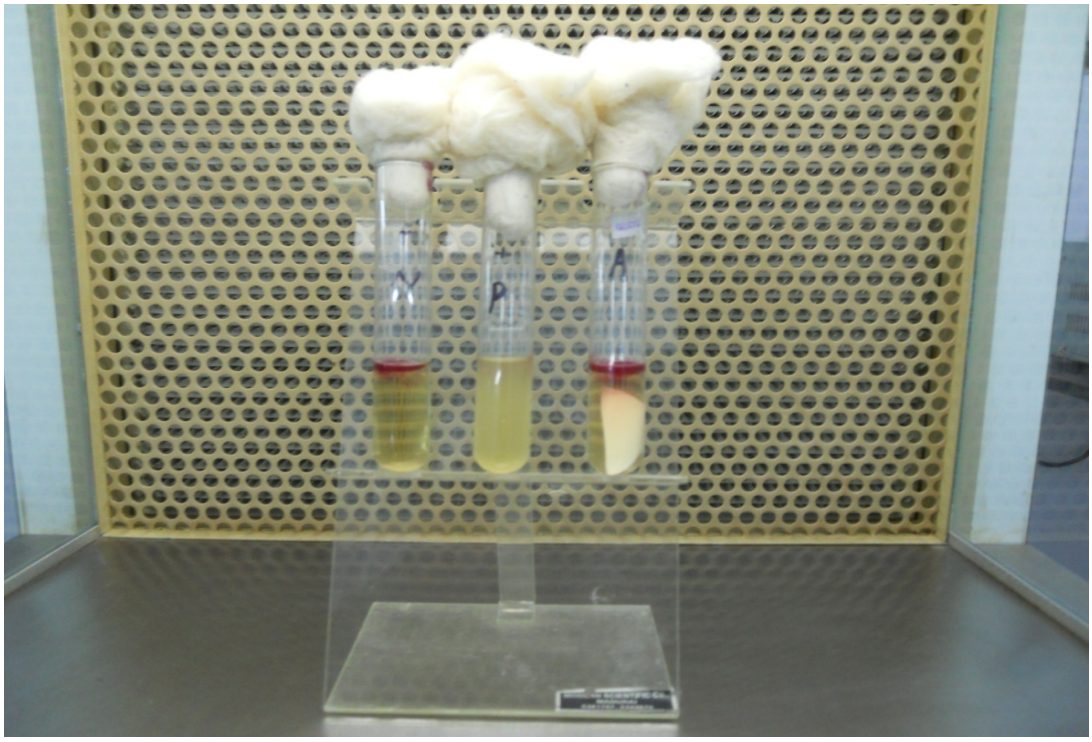


P – Positive control

N – Negative control

A - Sample

Fig:26 Observation of the sterility test done in fluid thioglycollate medium (FTM)



P – Positive Control

N – Negative Control

A - Sample

8. CONCLUSION

In this study niosomal drug delivery system was developed using non-ionic surfactant incorporating Zidovudine by Thin film hydration technique. The prepared niosomal vesicles were quite stable.

The formulation was subjected to Entrapment efficiency, Scanning electron microscopy, Invitro release, and Zeta potential analysis. From the results of experimental investigation, we concluded that, formulation F_{13} containing drug with 300:200 µmol (surfactant:cholesterol) ratio was showing higher percentage entrapment with desired sustained release of zidovudine. Hence formulation F_{13} was considered as optimized formulation.

Invitro release from optimized zidovudine niosomal formulation (F_{13}) showed extended release for 24 hours.

SEM image revealed the vesicles are exist spherical shape and uniform in size. Scanning electron micrograph shows there is no aggregation between the particles.

Negative zeta potential value was observed in zeta potential analysis. This confirmed the presence of negative charge inducing agent in formulation.

The formulation was checked for sterility as per I.P specification. The optimized formulation passes the sterility test.

Stability study was carried out for the period of three months at various storage conditions. The results showed that the formulation remains stable at 4°c.

The optimized formulation was found to follow zero order release pattern which was revealed by the linearity shown from the plot of Time Vs cumulative percentage drug release. From the drug release kinetic studies, we concluded that the drug was released from niosome by a zero order diffusion controlled mechanism.

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